T cell epitope mapping of JC polyoma virus-encoded proteome reveals reduced T cell responses in HLA-DRB1*04:01+ donors

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Abstract: JC polyomavirus (JCV) infection is highly prevalent and usually kept in a persistent state without clinical signs and symptoms. It is only during immunocompromise and especially impaired CD4(+) T cell function in the brain, as seen in AIDS patients or natalizumab-treated multiple sclerosis patients, that JCV may cause progressive multifocal leukoencephalopathy (PML), an often life-threatening brain disease. Since CD4(+) T cells likely play an important role in controlling JCV infection, we here describe the T cell response to JCV in a group of predominantly HLA-DR-heterozygotic healthy donors (HD) by using a series of overlapping 15-mer peptides spanning all JCV-encoded open reading frames. We identified immunodominant epitopes and compared T cell responses with anti-JCV VP1 antibody production and with the presence of urinary viral shedding. We observed positive JCV-specific T cell responses in 28.6% to 77.6%, humoral immune response in 42.6% to 89.4%, and urinary viral shedding in 36.4% to 45.5% of HD depending on the threshold. Four immunodominant peptides were mapped, and at least one immunogenic peptide per HLA-DRB1 allele was detected in DRB1*01(+), DRB1*07(+), DRB1*11(+), DRB1*13(+), DRB1*15(+), and DRB1*03(+) individuals. We show for the first time that JCV-specific T cell responses may be directed not only against JCV VP1 and large T antigen but also against all other JCV-encoded proteins. Heterozygotic DRB1*04:01(+) individuals showed very low T cell responses to JCV together with normal anti-VP1 antibody levels and no urinary viral shedding, indicating a dominant-negative effect of this allele on global JCV-directed T cell responses. Our data are potentially relevant for the development of vaccines against JCV.

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T Cell Epitope Mapping of JC Polyoma Virus-Encoded Proteome Reveals Reduced T Cell Responses in HLA-DRB1*04:01^{+} Donors

Running title: JCV-specific T cell epitope mapping in healthy donors

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Abstract

JC polyoma virus (JCV) infection is highly prevalent and usually kept in a persistent state without clinical signs and symptoms. Only during immunocompromise and especially impaired $CD4^+$ T cell function in the brain, as seen in AIDS patients or natalizumab-treated multiple sclerosis patients, JCV may cause progressive multifocal leukoencephalopathy (PML), an often life-threatening brain disease.

Since $CD4^+$ T cells likely play an important role in controlling JCV infection, we here describe the T cell response against JCV in a group of predominantly HLA-DR-heterozygotic healthy donors (HD) by using a series of overlapping 15-mer peptides spanning all JCV-encoded open reading frames. We identified immunodominant epitopes and compared T cell responses with anti-JCV VP1 antibody production and with the presence of urinary viral shedding.

We observed positive JCV-specific T cell responses in 28.6-77.6%, humoral immune response in 42.6-89.4% and urinary viral shedding in 36.4-45.5% of HD depending on the threshold. Four immunodominant peptides were mapped, and at least one immunogenic peptide per HLA-DRB1 allele was detected in $DRB1*01^+$, $DRB1*07^+$, $DRB1*11^+$, $DRB1*13^+$, $DRB1*15^+$, and $DRB1*03^+$ individuals. We show for the first time, that JCV-specific T cell responses may be directed not only against JCV VP1 and Large T antigen, but also against all other JCV-encoded proteins. Heterozygotic $DRB1*04:01^+$ individuals showed very low T cell responses to JCV together with normal anti-VP1 antibody levels and no urinary viral shedding, indicating a dominant-negative effect of this allele on global JCV-directed T cell responses. Our data are potentially relevant for the development of vaccines against JCV.

Introduction

JC virus (JCV) is a member of the polyomaviridae (PyVs) family (48), a small, DNA virus family, which encompasses eight more human viruses: BK PyV (BKV) (19), KI PyV (KIV) (2), WU PyV (WUV) (21), Merkel cell PyV (MCV) (17), HPyV6 and HPyV7 (51), trichodysplasia spinulosa-associated PyV (TSV) (62) and HPyV9 (52). JCV consists of a circular double-stranded DNA genome of 5130bp length and three capsid proteins, namely VP1, VP2 and VP3, with the major capsid protein VP1 being able to self-assemble into virus-like-particles (47), and a few additional proteins, agnoprotein, large T (LT) antigen, small T (ST) antigen and three T' antigens (T'135, T'136 and T'165) (18).

Infection with JCV is common in healthy individuals with IgG seroprevalence rates between 58% and 84% (15, 66). JCV entry into the organism might occur via infection of tonsillar tissue after inhalation or via fecal-oral route, but also by vertical transmission (6, 41). Usually, JCV infection remains clinically unapparent, and the virus persists in tonsils and hematopoietic precursor cells in the bone marrow. In a large fraction of infected individuals JCV also infects kidney epithelial cells and is associated with viral shedding in the urine in approximately 50% of individuals (35). The reasons for urinary viral shedding in only a fraction of infected individuals are not clear. Under circumstances of immunocompromise and especially impaired $CD4^+$ T cell function such as late-stage HIV infection, hematological malignancies, organ transplantation, but also in clinically inconspicuous idiopathic $CD4^+$ lymphopenia, JCV is able to cause an opportunistic infection of the brain, PML (35, 58). PML is caused by infection of oligodendrocytes and astrocytes by neurotropic JCV strains with altered regulatory regions and often certain amino acid exchanges in the JCV major capsid protein VP1 when compared to archetypic strains (24, 27). Cell lysis of oligodendrocytes leads to widespread demyelination, serious neurological impairment with fatal outcome in 30 to 50% of cases (35, 58). Besides the abovementioned causes, PML has become a serious concern during therapy with a few monoclonal antibodies, e.g. MS patients receiving the highly effective and usually well-tolerated antibody against α-4-integrin (VLA-4; CD49d), natalizumab, but also in systemic lupus erythematosus and psoriasis

patients receiving anti-CD20 and anti-LFA-1 antibodies respectively (35, 58). Until now 285 out of 104,400 natalizumab-treated MS patients have developed PML in the post-marketing setting worldwide, and approximately 22% have died from the complication (61). Current risk estimates vary between 2.33:1000 and 2.95:1000 in MS patients on natalizumab therapy, but rise to approximately to 9:1000 or higher in JCV seropositive individuals with more than 2 years treatment and prior immunosuppression (61). The treatment complication of PML therefore threatens to lead to serious regulatory restrictions or even market withdrawal.

JCV-specific immunity is probably not only important for containing JCV infection in healthy individuals, but also for recovery from PML, since immune reconstitution is associated with the best clinical outcome among patients with PML (8, 13, 37). Serum antibodies against JCV are frequent in clinically healthy individuals, whereas intrathecal JCV-specific antibodies are found at high prevalence and high titers in PML patients (66). Furthermore, JCV-specific IgG increases during PML disease course of PML survivors (30). However, as most PML patients harbour JCV-specific antibodies before or at onset of the disease (5), the humoral immune response may not be sufficient to prevent the development of PML. JCV-specific, cellular immune responses have been investigated as well, particularly the role of $CD8^+$ cytotoxic T cells (12, 31). In individuals expressing the common human leukocyte antigen (HLA) class I molecule, (HLA-) A*02:01, two nonamer peptides have been identified as immunodominant JCV peptides: VP1 (100-109) peptide (ILMWEAVTL) and VP1 (36-44) peptide (SITEVECFL) (12, 31). The frequency of $CD8^+$ T cells specific for these epitopes varies between 1:100,000 to 1:2,494 in peripheral blood mononuclear cells (PBMC), and 73% of immunocompetent individuals possess such cells (14). JCV-specific $CD8^+$ T cells have been shown in 91% of PML survivors, while they are only detectable in 9% of PML progressors (31). Furthermore, the number of JCV-specific $CD8^+$ T cells at the onset of the disease can predict subsequent disease progression. Based on these data it has been concluded that the JCV-specific $CD8^+$ cytotoxic T cell response is essential for the prevention of and resolution from PML.

Despite the forecited experience of an increased risk of PML in AIDS patients with low $CD4^+$ T levels, and in patients with idiopathic $CD4^+$ lymphocytopenia, both

suggesting an important role of JCV-specific $CD4^+$ T cells in the control of JCV infection, $CD4^+$ T cell function has been examined in fewer studies. Peripheral $CD4^+$ T cell counts below 200 cells/µl at the time of PML diagnosis were found associated with progression and lower survival rate at one year compared to $CD4^+$ T cell counts above 200 cells/µl (37). $CD4^+$ T cells reactive to JCV-like particles have been detected in healthy and HIV-infected non-PML individuals with urinary excretion of JCV (20). Furthermore, HIV-infected PML survivors harbour $CD4^+$ T cells reactive to JCV-like particles, while they are absent in HIV-infected individuals with active PML (20). With 80% of PML patients suffering from AIDS as underlying disease, and reciprocally up to 5% of AIDS patients developing PML (35, 58), $CD4^+$ T cells are likely of particular importance in the control of JCV. Moreover, like antibody-producing B cells, $CD8^+$ T cells are at least in part dependent on functional help by $CD4^+$ T cells. Supporting this notion we have recently demonstrated that JCV-specific $CD4^+$ T cells of T helper 1 (T_h1) or T_h1-2 phenotypes predominantly infiltrate the brain in PML-immune reconstitution inflammatory syndrome (PML-IRIS), a condition occurring for example upon washout of natalizumab in MS or restoration of $CD4^+$ numbers in AIDS patients (3). While PML-IRIS and the accompanying inflammation and swelling of the brain are also life-threatening, it leads to the elimination of JCV infection and PML and underscores the role of $CD4^+$ T cell responses.

In the present study we used a series of overlapping peptides that span all six open reading frames of JCV including the 35 most frequent variants of JCV and characterized the proliferative response in 49 healthy donors (HD). We subsequently identified immunodominant epitopes out of the four most immunogenic pools. As expected and consistent with the fact that our strategy primarily tested $CD4^+$ T cells we found that the responses depend on the HLA-class II haplotype with respect to peptide specificity and magnitude of the response. Of particular note, HLA-DRB1*04:01^+ donors showed low to absent proliferative responses to JCV peptides, were negative for urinary JCV viral DNA, but produced large amounts of anti-JCV VP1 antibodies. JCV-specific T cell responses were also reduced in individuals expressing DRB1*01:01. Our data are of interest in the context of vaccine development and studies of the cellular immune responses against JCV under physiological and pathological conditions.

Material and Methods

Donors

49 HD were recruited from the Blood Bank at the University Medical Center Hamburg-Eppendorf, Germany, forming cohort 1 (Table 1). The mean age of the donors was 46.5 years (range 23 to 72 years), and the gender distribution was 28 females and 21 males. Cohort 1 was used to determine the peripheral cellular response to JCV peptide pools, individual peptides and JCV VP1 virus-like-particles (VLP). The humoral response to JCV VLP was assessed by testing the supernatant fluids obtained after Ficoll density centrifugation in this cohort, since we did not have access to serum samples from these donors. Cohort 2 contains 22 HD recruited from our institute staff and with a mean age of 35.4 years (range 26 to 46 years) (Table 1). The gender distribution in cohort 2 was 15 females and 5 males. Cohort 2 was employed to investigate urinary viral shedding and the relation between T cell responses and the presence of JCV-specific IgG antibodies in serum. The study was approved by the local ethics committee (Ethik-Kommission der Ärztekammer Hamburg, No. 2758), and informed consent was obtained from all study subjects.

HLA typing

All individuals in cohort 1 and 2 were typed for HLA class II molecules at the HLA laboratory, University Medical Center Hamburg-Eppendorf, Germany. Isolation of DNA was performed with the help of QiaCube™ (Qiagen GmbH, Hilden, Germany) from whole blood with a final concentration of 30 ng/µl. The samples were high resolution typed for HLA class II (DRB1*, DRB3*, DRB4*, DRB5*, DQA1* and DQB1*) with a sequence-specific oligonucleotide (SSO)-based test Dynal-RELIT™-SSO (Invitrogen GmbH, Karlsruhe, Germany) and with the sequencing-based typing kit AlleleSEQR™ HLA-SBT (Abbott Molecular, Wiesbaden, Germany). HLA class II types are listed in Table S1 (cohort 1) and Table S2 (cohort 2). Frequencies of HLA class II alleles among cohort 1 and 2 are summarized in Table S3.

183 **JCV sequence analyses**

184 JCV sequences available in all databanks were retrieved and analyzed by using
185 the HUSAR software package (53). The amino acid sequences of all proteins (VP1,
186 VP2, VP3, agnoprotein, LTAg and STAg) from all nine human polyomaviruses were
187 extracted from reference genomes available in GenBank, i.e. BKV (accession number
188 NC_001538), JCV (NC_001699), KIV (NC_009238), WUV (NC_009539), MCV
189 (NC_010277), TSV (NC_014361), HPyV6 (NC_014406), HPyV7 (NC_014407), HPyV9
190 (NC_015150). Each of the corresponding proteins derived from all human polyomavirus
191 reference genomes were aligned with the ClustalW program (60). Furthermore, 479
192 VP1 amino acid sequences were aligned. Conservation of the sequence alignments
193 was plotted with the Plotcon program from the EMBOSS software package (50) by
194 using a window size of 25. All multiple sequence alignments can be provided by the
195 authors upon request. Maximum sequence similarity of immunodominant JCV peptides
196 was calculated by using epitope conservancy analysis from the IEDB and analysis
197 resource (www.immuneepitope.org) (65).

198

199 **Proteins and peptides**

200 For the identification of JCV-specific $CD4^+$ T cells, 204 (13-16-mer) peptides
201 covering the entire JC viral proteome (Fig. 1A) were applied. Peptides were synthesized
202 and provided by pe (peptides and elephants GmbH, Potsdam, Germany). The peptides
203 overlap by 5 amino acids and include 35 peptides with common single amino acid
204 mutations (Table S4). To account for amino acid variations (Fig. 1B), that occur among
205 the different JCV genotypes and strains, amino acid sequences of each JCV-encoded
206 protein including agnoprotein (Agno), VP1, VP2, VP3, large T antigen (LTAg), small T
207 antigen (STAg) and three T' antigens (T'135, T'136 and T'165) from all 479 JCV
208 genomic sequences available in GenBank (by March 2008) were aligned with the
209 ClustalW program (60) and those polymorphisms, which were prevalent in more than
210 1% of all retrieved sequences, were defined as common mutations. Peptides were
211 arranged in a set of 42 pools, each pool containing 5 different peptides (Fig. 1C, Table
212 S5). Variations of the same peptide were not arranged in the same pool.

JCV VP1 protein forms virus-like (VLP) particles, and VP1 and VLP are therefore used as interchangeable terms. VP1 protein forming VLP (VP1/VLP) was generated by the Life Science Inkubator, Bonn, Germany, as previously described (22). Tetanus toxoid (TTxd) (Novartis Behring, Marburg, Germany) was used as positive control.

Proliferation and activation assays

Peripheral blood mononuclear cells (PBMC) were separated by Ficoll density gradient centrifugation (PAA, Pasching, Austria), always using the same volume/volume ratio of blood/Ficoll. Proliferation against peptide pools was performed with fresh PBMC and proliferative assays with individual peptides with frozen cells from the same individuals from the same date of sampling. In both assays T cell responses to VLP, TTxd and PHA were also analyzed. 2 x 10^5 PBMC per well were seeded in 96-well U-bottom microtiter plates in RPMI (Invitrogen, California, USA) containing 2 mM glutamine, 50 µg/ml gentamicin, 100 U/ml penicillin/streptomycin (all from Whittaker Bioproducts, California, USA) and 5% pooled human AB serum. JCV peptide pools, JCV individual peptides, VLP or TTxd were added to the cultures and cells were cultured for seven days. PHA-L (1 µg/ml, Sigma, St Louis, MO) was added to some wells on day four. Wells containing only cells and medium served as negative controls. All JCV peptides were either tested in pools at a final concentration of 2 µM per peptide or as individual peptides at a concentration of 10 µM. TTxd was tested at 5 µg/ml. To detect frequencies of JCV-specific cells lower than 1 in 2 x 10^5 PBMC, 8 replicate wells were seeded for each peptide pool. Individual peptides were tested by seeding 10 wells per peptide. To control for variability between 96-well U-bottom microtiter plates in each HD, each plate contained wells stimulated with JCV peptides, PHA, TTxd and VLP, as well as unstimulated wells.

After seven days of incubation, proliferation was measured by ^{3}H-thymidine (Hartmann Analytic, Braunschweig, Germany) incorporation in a scintillation beta counter (Wallac 1450, PerkinElmer, Rodgau-Jürgesheim, Germany). The stimulation index (SI) was calculated as SI = counts per minute (cpm) of an individual well with antigen / mean cpm of 72 negative control wells. Individual wells with peptide pools

were considered positive if SI was >3 (Fig. 2A). In order to detect reactivity of thawed cells against single peptides we considered positive individual wells, with SI > 2.

To determine, whether preferentially $CD4^+$ or $CD8^+$ T cells or both of these T cell subsets equally were stimulated by the above described JCV-derived 13-16-mer peptides, representative blood samples from cohort 1 were analyzed by using carboxyfluorescein succinimidyl ester (CFSE, Sigma, St Louis, USA) dye dilution for proliferation and by intracellular cytokine staining (ICS) using interferon-γ (IFN-γ) for activation. Briefly, 10^7 PBMCs/ml were incubated with 0.5 µM CFSE for eight minutes at room temperature, labeling was stopped by 33.3% fetal calf serum and unbound CFSE was washed out. 2 x 10^5 CFSE-labeled PBMC per well were seeded in 96-well U-bottom plates in above mentioned medium. For each donor, two JCV peptide pools, which had been identified as immunodominant in the respective donor by ^{3}H-thymidine incorporation assays, were selected for stimulation and, for each peptide pool, 20 wells were stimulated at a final concentration of 2 µM per peptide. Six wells were plated without antigen to serve as negative control. TTxd at 5 µg/ml served as positive control. After 10 - 14 days, five JCV peptide-stimulated wells were pooled and processed for IFN-γ ICS. Therefore, the respective wells were restimulated with the corresponding JCV peptide pool at a final concentration of 2 µM for each peptide, and after 1h of incubation at 37°C Golgistop (BD Biosciences, Heidelberg, Germany) was added, followed by incubation for 5 h at 37°C. After washing twice with PBS, cells were labeled with Live/Dead Fixable Aqua Dead Cell Stain Kit (Invitrogen, GmbH, Karlsruhe, Germany). After blocking cellular Fc receptors with human IgG (Sigma, St Louis, USA), following surface markers were stained with antibodies: CD3 (PE, clone SK7, Biolegend, London, UK), CD4 (APC, clone RPA-T4, eBioscience, San Diego, USA) and CD8 (PB, clone DK25, Dako, Glostrup, Denmark). Cells were fixed and permeabilized with Cytofix/Cytoperm (BD Biosciences) according to manufacturer's protocol, washed, and stained with an anti-IFN-γ antibody (PE-cy7, clone 4S.B3, BD Biosciences). Cells were washed twice with 200 µl FACS buffer (0.01% sodium azide and 1% bovine serum albumine in PBS) before samples were analyzed with a LSR-II flow cytometer (BD Biosciences) and FACS Diva Software (BD Biosciences), gating on lymphocytes, singlet cells, live cells, $CD3^+$ cells, $CD4^+$ and $CD8^+$ cells, $CFSE^{dim}$ and IFN-γ^+ cells (Fig. S1A).

Approximately 30,000 singlet, live, $CD3^+$ lymphocytes were recorded per sample. The stimulation index for CFSE labeled cells was calculated as SI [CFSE] = percentage of $CFSE^{dim}$ T cells among a pooled set of five wells with antigens / percentage of $CFSE^{dim}$ T cells among a pool of six unstimulated control wells (Fig. 2B and Fig. S1B). The stimulation index for IFN-γ ICS was calculated as SI [IFN-γ] = percentage of IFN-γ^+ T cells among a pooled set of five wells with antigens / percentage of IFN-γ^+ T cells among a pool of six unstimulated control wells (Fig. 2B and Fig. S1B).

ELISA for VP1/VLP-specific antibodies

The level of JCV VP1/VLP-specific IgG antibodies was determined as described previously (66). Briefly, ELISA plates were coated with 100 µl JCV VLP (1 mg/ml)/well and incubated with serum samples or Ficoll supernatant, diluted 1:5000 in blocking buffer (5% non-fat powdered milk in 25mM Tris 150mM NaCl), and serial dilutions of a positive control. Human IgG was captured by a biotin-conjugated anti-human Fc antibody (eBioscience, Frankfurt, Germany) and detected by an avidin horseradish peroxidase (eBioscience, Frankfurt, Germany). Extinction was measured as optical density at 450 nm (OD_{450}) using a µQuant microplate reader (Perkin Elmer, Waltham, MA, USA). The measured extinctions were normalized to the OD_{450} of the positive control on the plate and reported as normalized OD_{450} (nOD_{450} = mean OD_{450} of sample replicates / mean OD_{450} of positive control replicates on the plate). The same human serum was always used as positive control and its reactivity approximated an OD_{450} of 1. To check comparability between Ficoll supernatants and sera, JCV-specific antibody levels were measured in sera and Ficoll supernatants of three healthy donors, from whom sera and whole blood for Ficoll gradient centrifugation was collected at the same time. Ficoll supernatant showed reproducibly a dilution factor of 1.2 in comparison to the serum sample from the same donor.

In order to determine serological cross-reactivity between JCV and BKV VP1 proteins (kindly provided by Natalie Meinerz and Robert Garcea, Department of Molecular, Cellular, and Developmental Biology, University of Colorado Boulder, USA), representative serum samples underwent competitive inhibition (“pre-adsorption”)

assays. Specifically, serial dilutions of BKV VP1 and JCV VP1 proteins were added to a 1:5000 dilution of serum in blocking buffer and incubated for 1 h at 4°C prior to incubating samples with immobilized JCV VP1 and completing the ELISA as described previously (66). The concentration needed to sufficiently and reliably compete VP1-specific antibodies was 4.86 µg/ml. Percent inhibition (PI) was calculated as PI = 100 x [1 - (average nOD_{450} VP1 pre-incubated samples / average nOD_{450} buffer incubated samples)].

Quantitative PCR assay for JCV detection

The JCV DNA load was quantified in urine by applying a quantitative PCR (qPCR) with a set of primers and probe located at the T antigen of JCV (49). Viral DNA was extracted from 750 µl of urine by applying the QIAampUltraSens Virus Kit (Qiagen, Hilden, Germany). Nucleic acids were recovered in 60 µl of elution buffer. Thus, in a qPCR reaction, 125 µl of urine were analyzed. The assay was specific for JCV detection and the lower limit of JCV DNA detection was 1-10 copies per reaction, with a linear range from 10^2 to 10^7 genome copies per reaction. Amplifications were performed in a 25 µl reaction mixture containing 10 µl of sample and 12.5 µl of TaqMan® Environmental Master Mix 2.0 (Applied Biosystems, Madrid, Spain) which offers accurate, real-time PCR-based pathogen detection and is highly sensitive even in the presence of inhibitors. The concentration of each primer (JE3R and JE3F) was 0.9 µM and fluorogenic probe (JE3P) 0.225 µM. This primer/probe set is specific for JCV and does not detect human polyomaviruses BK PyV nor SV40 or bovine polyomavirus (49). Following activation of the AmpliTaq Gold for 10 min at 95ºC, 40 cycles (15s at 95ºC and 1 min at 60ºC) were performed in aMx3000P detection system (Stratagene, Santa Clara, USA). Neat and a ten-fold dilution of the samples were run in duplicates (4 runs/sample), whereas each dilution of standard DNA suspension (from 10^2 to 10^7) was run in triplicate. In all qPCR assays the amount of DNA was defined as the mean of the data obtained. A non-template control (NTC) was added to each run in addition to the negative control of the nucleic acid extraction. The possible presence of inhibitors, potentially leading to false negative results, has been tested adding low copy numbers

of viral DNA to urine samples. JCV Mad1 cloned into the EcoRI site of a pBR322 vector was used for the standard curve. All qPCRs were done in different rooms isolated from post-PCR samples to prevent contamination.

Results

JCV-specific immune response in healthy donors

In order to study the $CD4^+$ T cellular immune response to JCV, 204 13-16-mer peptides overlapping by 5 amino acids and spanning all JCV proteins (Agno, VP1, VP2, VP3, LTAg, STAg and T' antigens T'135, T'136 and T'165) were synthesized and arranged in 42 pools (Fig. 1A-C, and Table S5). PBMC from 49 HD (cohort 1) were tested for antigen-specific proliferation against the 42 pools. T cell responses showed a high degree of inter-individual variability with respect to recognition of different peptide pools, the magnitude of the proliferative response, i.e. the stimulation indices of individual proliferating wells, and also with respect to the number of positive wells responding to a single pool (Fig. 2A). Each of the above measures contains different information about JCV-specific T cell responses. First, the number of different pools that elicit a proliferative response in a given donor expresses whether the JCV-specific T cell response is broad, when many peptide pools are recognized, or focused, if only a few or one pool are positive. Second, the magnitude of the response (i.e. stimulation index) expresses the amount of incorporated thymidine and hence indirectly the number of cell divisions. And third, since a defined number of PBMC is seeded per well, the number of positive wells per pool serves as a measure of the presence in a predefined amount of cells serving as a very rough approximation of the precursor frequency of antigen-specific T cells. These 3 measures together allow assessing the JCV-specific immune response within a single individual. Regarding our data, PBMC from some of the HD failed to proliferate against any of the peptide pools (Fig. 2A, upper graph), while others showed a strong proliferative response against multiple peptide pools (Fig. 2A, lower graph). The mean SI of negative control wells and tetanus toxoid (TTxd)-stimulated wells of the same individuals are shown in the right graphs. CFSE labeling and IFN-γ ICS of PBMCs from representative donors confirmed, that $CD4^+$ T cells, and not $CD8^+$ T cells, preferentially responded to stimulation with donor-specific immunodominant JCV peptide pools as identified by ^{3}H-thymidine incorporation assays (Fig. 2B and Fig. S1B). $CFSE^{dim}$ cells representing proliferated cells were predominantly activated upon restimulation with JCV peptides as shown by IFN-γ secretion in the ICS, and IFN-γ

secreting cells were predominantly $CFSE^{dim}$. Since ^{3}H-thymidine incorporation assays are more convenient for the analysis of such a high number of peptide pools and donors, we decided to analyze the fine-specificity of JCV-specific $CD4^{+}$ T cells in-depth by using ^{3}H-thymidine incorporation assays.

We also analyzed the JCV-specific humoral response and shedding of JCV DNA in urine. VLP-specific antibodies were quantified by ELISA in 44 HD. JCV DNA was determined by PCR in urine from 22 HD. JCV-specific humoral responses and shedding of JCV DNA in urine also showed a high degree of inter-individual variability (Fig. 2C and D). Considering a dilution factor of 1.2 for Ficoll supernatant related to serum, nOD_{450} values among Ficoll supernatants of cohort 1 and sera of cohort 2 showed similar variability (Fig. 2C). Displayed are the urinary JCV DNA copy numbers of all 10 JCV-excreting individuals (Fig. 2D).

Next, in order to examine whether JCV-specific T cellular immune responses are indicative of JC virus infection, we calculated the percentage of HD with positive T cell response to JCV and compared this value with the percentage of HD presenting VLP-specific antibodies and with JCV DNA in urine. The latter are both parameters currently used to identify JCV-infected individuals. To calculate the percentage of HD with positive JCV-specific T cell responses we used two different arbitrary thresholds for positivity. The low threshold considers that a HD has a positive JCV T cell response, if she/he shows 3 or more wells with SI ≥ 3, while the high threshold considers 10 or more wells with SI ≥ 3. Analogous thresholds were used to determine the percentage of HD with VLP-specific antibodies (low threshold at $nOD_{450} \geq 0.08$; high threshold at $nOD_{450} \geq 0.2$) and with JCV DNA in urine (low threshold at copy number ≥ 10 GC/ml; low threshold at copy number ≥ 100 GC/ml). Using the low thresholds for positivity, 77.6% of the HD were positive for JCV-specific T cell response compared to 89.4% for anti-VLP antibodies, and only 45.5% of HD shedding JCV DNA in urine. Using the high thresholds 28.6% of HD were positive for JCV-specific T cell response, 42.6% for anti-VLP antibodies and 36.4% for JCV DNA in urine. Considering a low threshold of $nOD_{450} \geq 0.08$ for the definition of seropositive donors, 33.3% of seropositive individuals showed no T cell proliferation to JCV peptides (defined as less than 3 wells with SI ≥ 3). 45,5% of seropositive donors showed no T cell proliferation to JCV peptides, when a

high threshold of nOD_{450} ≥ 0.2 was applied. The association between JCV-specific antibody level and urinary JC viral load was weak (correlation coefficient r=0.27), similar to the results published by Gorelik et al. (23). As a result of the use of two cohorts, measures of all the three parameters in the same HD that would allow us to study correlations are not available. This was due to using healthy blood bank donors, from whom serum and urine could not be obtained.

Identification of JCV immunodominant epitopes

First, we analyzed T cell responses from the 49 HD of cohort 1 against the 42 peptide pools. Individuals responded to peptide pools from all JCV proteins (Fig. S2). The preference and strength of reactivity against peptide pools from a specific JCV protein varied between HD (Fig. S3). Most donors recognized pools derived from four different JCV proteins (36.7%), some donors recognized three different proteins (20.4%), and only 12.2% of all HD recognized peptide pools derived from all JCV-encoded proteins.

To determine the JCV peptide pools that likely contained immunodominant peptides, we performed a detailed analysis of T cell responses of cohort 1. Responses of individual wells were considered positive when the SI was higher than 3. First, the sum of SIs (∑(SI>3)) from positive wells was calculated to account for strength and proliferative capacity of each donor to each peptide pool (Fig. 3A, upper graph). To correct for background proliferation, we calculated the ∑(SI>3) also for unstimulated wells, normalized the value for the number of wells and subtracted it from the ∑(SI>3) corresponding to each pool and donor. Next, in order to integrate an estimation of the precursor frequency of JCV-peptide-specific T cells, we calculated the fraction of positive wells (number of total positive peptide wells (SI>3) per number seeded wells) (Fig. 3A, middle graph). Again, with the purpose to exclude background proliferation, the fraction of unstimulated wells with SI>3 was calculated, normalized and subtracted from the fraction of positive peptide-stimulated wells. Finally, we determined as a third indicator of immunogenicity the frequency of donors with at least one positive well for each pool (Fig. 3A, lower graph). Using these three parameters we created a reactivity

score (RS = ∑(SI>3) x percentage of reactive wells x percentage of reactive individuals) for each pool that takes into account the stimulatory capacity of the pool, the frequency of peptide-specific cells in the pool and the frequency of donors with at least one well positive for the pool (Fig. 3B). Using this approach we identified pool 14 (containing VP1 peptides), 17 (containing VP2 peptides), 32 and 33 (containing LTAg peptides) as the pools showing higher RS and in consequence to be the best candidates to contain immunodominant peptides. These pools had a ∑SI>3 higher than 125 with a percentage of reactive wells of at least 5.0% and recognition by more than 30% of HD.

Although the use of 15-mer peptides in combination with the settings of the proliferative assay favors detection of $CD4^+$ T cell responses according to current knowledge, we cannot exclude contribution of $CD8^+$ T cells in the proliferative response to pools 14, 17, 32 and 33 since 15-mer peptides can be shortened by proteolytic cleavage and presented on HLA class I molecules.

To identify immunodominant JCV peptides, we tested PBMC from 20 HD (cohort 2) with the 20 individual peptides contained in pools 14, 17, 32 and 33. Reactivity scores for each individual peptide were calculated as mentioned above, but considering positive a response with SI>2, since we used fewer cells per plate (100.000/well) due to a limited amount of cells and cells had been frozen before their use. Accordingly, a lower proliferative capacity of the cells enrolled in these assays along with correspondingly less background proliferation appeared. Results are summarized in Fig. 3C. We identified peptide 41 (VP1 (123-137) variant 3; V3) as the immunodominant peptide contained in pool 14, and peptides 81 (VP2 (30-43)) and 82 (VP2 (39-51)) as the immunodominant peptides contained in pool 17. Pool 33 contained the immunodominant peptide 162 (LTAg (415-429)). We were not able to identify an immunodominant peptide in pool 32.

HLA class II restriction of JCV-specific cellular immune responses

$CD4^+$ T cells recognize antigenic peptides in the context of HLA class II molecules. Table S1 shows the HLA class II alleles of the 49 HD from cohort 1. HD were divided into twelve groups depending on HLA-DR expression (Table S3). Group

DR1 included all donors expressing the HLA-DRB1*01 alleles (n=8), and similarly we grouped donors expressing HLA-class II molecules of the major subtypes DRB1*04 (n=15, DR4), DRB1*07 (n=13, DR7), DRB1*08 (n=2, DR8), DRB1*10 (n=1, DR10), DRB1*11 (n=9, DR11), DRB1*12 (n=4, DR12), DRB1*13 (n=12, DR13), DRB1*14 (n=1, DR14), DRB1*15 (n=13, DR15), DRB1*16 (n=7, DR16), and DRB1*03 (n=10, referred to as DR17). The circle diagram in Fig. 4A represents the frequency of the most common DR types within this HD cohort. It is important to note, that nearly all donors were heterozygote for HLA-DRB1*alleles, and are therefore contained in two different HLA-DR groups. Proliferative responses were analyzed for HLA-DRB1* cohorts, which were represented by at least 8 individuals. To analyze the T cell response of each HD group against the 42 pools we calculated the RS as described above. Because each group was made up by different numbers of HD, we normalized each group for the number of donors, therefore, values are directly comparable and adjusted to n=10. PBMC from HD expressing different HLA class II molecules responded to different peptide pools (Fig. 4B) and also the RS varied considerably among groups. HD from groups DR7 and DR17 showed significantly higher RS to JCV peptides, than HD from groups DR1 and DR4, while no differences were observed in RS to TTxd (Fig. 4C and D). The preference for specific JCV proteins also varied among HLA-DR groups (Fig. S2 and Fig. S3). All HD except of $DR13^+$ and $DR16^+$ HD recognized peptides from the amino-terminal region of VP2 more often than expected (Fig. S2). Peptides from the carboxy-terminal region of VP2 were recognized less frequently than expected (Fig. S2). $DR8^+$, $DR12^+$ and $DR13^+$ HD recognized peptides from the LTAg more often than expected (Fig. S2). Importantly, the preference for specific JCV proteins was also variable within a group expressing certain HLA alleles (Fig. S3), which is most likely due to the fact that there is variability with respect to the second HLA-DR allele expressed by the heterozygote donors. The pools inducing the strongest cellular response in the entire cohort (pools 14, 17, 32 and 33) were pools that were stimulatory in the context of several class II alleles including the two groups showing the strongest responses (group DR7 and group DR17) (Fig. 4B).

Next, we examined whether the peptides identified as immunodominant after analyzing the whole HD cohort were also immunodominant for all HLA-DR haplotypes.

PBMC from cohort 2 expressing different HLA-class II alleles (Fig. 5A) were tested against the 20 peptides contained in the pools inducing the strongest cellular response in the entire cohort (pools 14, 17, 32 and 33). The reactivity scores induced by these 20 peptides are summarized in Fig. 5B. The peptides inducing the highest RS clearly differed between HLA subgroups showing that HLA class II alleles contribute to the fine specificity of $CD4^+$ T cells against JCV peptides. An important factor in determining immunodominance is binding to the respective HLA-class alleles, which can be predicted *in silico* by a number of publicly accessible databases. When we calculated the *in silico*-predicted binding scores by using the consensus method from the IEDB Analysis Resource (65), the binding scores of immunodominant JCV peptides varies broadly depending on the HLA allele (Table S6). Peptide 41 (VP1 (123-137) V3) was identified as immunodominant peptide in the whole cohort, probably because it was strongly stimulatory in DR11 and DR13 donors, who represented 50% of all tested individuals. Similarly, peptide 82 (VP2 (39-51)) was identified as immunodominant, most likely because it induced prominent responses in individuals expressing DR7 and DR17. Sequences for all immunodominant JCV peptides are summarized in Table 2. All peptides identified as immunodominant except for the two JCV VP1-derived peptides showed highest sequence similarity with BKV-encoded protein sequences (mean similarity 78.4%, range 53.3 – 100%), when compared to all human PyV (Table 2). BKV-derived peptides previously described as immunodominant and/or cross-reactive to JCV were found to match to peptides from immunodominant pools 32 and 33 (LTAg) and peptides from substantially recognized pools 4, 5, 6, 35 and 38 of the present study (Table S7 and Table S8).

Reduced cellular JCV-specific T cell immune response in DRB1*04:01$^+$ donors

A more detailed analysis of HD from the DRB1*04^+ group revealed marked differences in JCV-specific T cell responses. The RS of HD expressing the DRB1*04:01 allele were significantly lower than those of HD expressing other DRB1*04 alleles (Fig. 6A and B) or non-DR4 alleles (Fig. 4C). Interestingly, this reduced T cell responsiveness was

519 “specific” for JCV, since the response to the recall antigen TTxd in these donors was
520 comparable to those of HD expressing other DRB1*04 molecules (Fig. 6C).

521 In order to examine further the reduced JCV T cell response in DRB1*04:01$^+$
522 donors, we determined the presence of JCV DNA in urine samples from eight
523 DRB1*04:01$^+$ donors, from seven expressing other DR4 alleles and from 10 HD not
524 expressing any DR4 allele. Results are summarized in Fig. 6D. Remarkably, none of the
525 urine samples from HD expressing the DRB1*04:01 allele tested positive for JCV DNA.
526 In contrast, JCV DNA was detected in 4 out of 5 (80.0%) urine samples from HD
527 expressing DR4 alleles other than DRB1*04:01 and in 6 out of 10 (60.0%) from donors
528 not expressing DR4 alleles (Fig. 6D). These results together with the low frequency of
529 JCV-specific T cells in DRB1*04:01$^+$ HD suggested that these individuals might not
530 have been exposed to the virus. To examine this issue further, we then tested the
531 humoral response against JCV VP1 by ELISA to assess their infectious state in serum
532 samples from seven DRB1*04:01$^+$ donors, five HD expressing DR4 alleles other than
533 DRB1*04:01 and from 10 HD not expressing any DR4 allele. DRB1*04:01$^+$ donors
534 showed similar or even higher levels of anti-JCV antibodies compared to the two other
535 groups (Fig. 6E). Serological cross-reactivity against BKV VP1 was excluded by
536 competitive pre-adsorption assays (Fig. 6F). Reactivity of serum samples from five
537 DRB1*04:01$^+$ donors was efficiently inhibited by pre-incubation with 4.86 µg/ml JCV
538 VP1 (median PI, 64.9%; interquartile range [IQR], 58.5 to 68.6%; minimum inhibition,
539 52.1%) and was minimally inhibited by 4.86 µg/ml BKV VP1 (median PI, 1.4%, IQR, 0.0
540 to 2.2%; maximum inhibition, 3.0%). Similarly, serum samples from seven DRB1*04:01$^-$
541 donors, including three HD expressing DR4 alleles other than DRB1*04:01, showed
542 strong inhibition after pre-incubation with 4.86 µg/ml JCV VP1 (median PI, 83.7%;
543 interquartile range [IQR], 68.3 to 88.2%; minimum inhibition, 52.2%), but minimal
544 inhibition after pre-adsorption with 4.86 µg/ml BKV VP1 (median PI, 1.5%, IQR, 0.0 to
545 6.9%; maximum inhibition, 12.3%). Using a threshold of $nOD_{450} \geq 0.08$ for the definition
546 of seropositive HD, 27.8% of DRB1*04:01$^-$ donors and 44.4% of DRB1*04:01$^+$
547 individuals showed no T cell proliferation to JCV peptides (less than 3 wells with SI ≥ 3
548 per donor). Using the higher threshold for seropositivity ($nOD_{450} \geq 0.2$), even 60% of
549 DRB1*04:01$^+$, but only 33.3% of DRB1*04:01$^-$ donors showed no T cell proliferation.

550 The anti-JCV VP1 seropositivity strongly argues against the hypothesis that these
551 donors were not exposed to JCV, but, despite the weak $CD4^+$ T cell responses against
552 JCV peptide pools, showed a robust antibody response against the major capsid protein
553 from as yet unknown mechanisms

554

555 **Reduced cellular JCV-specific immune responses in DRB1*01:01$^+$ healthy donors**

556 Similar to DRB1*04:01-heterozygous donors, HD positive for DRB1*01$^+$ mounted
557 significantly weaker JCV-specific cellular responses than $DR7^+$, $DR15^+$ and $DR17^+$ HD
558 (Fig. 4B and 5C), although cellular reactivity against TTxd did not differ among these
559 groups (Fig. 4D). Unfortunately, we could not analyze urinary shedding or JCV-specific
560 humoral response in $DR1^+$ HD because of lack of samples.

561

Discussion

In the present study we examined the cellular immune response against JCV with respect to prevalence of positive $CD4^+$ T cell responses, specificity for JCV proteins/peptides and relation to HLA-class II haplotype of the donor to develop a better understanding of JCV-specific immune control, to identify potential risk factors for PML and as a basis for future vaccination approaches. We identified several immunodominant peptides within the most prominent peptide pools, peptide 41 (pool 14), peptides 81 and 82 (pool 17), peptide 155 (pool 32), and peptide 162 in pool 33 (Fig. 3C). However, after stratification for HLA-class II haplotypes differences in the responses to peptides pools as well as to individual peptides within these pools became visible (Fig. 4). While the magnitude of the response to the pools differed for example between individuals carrying DR15 and DR17 alleles versus those carrying DR1 and even more so DR4 alleles, the focus of the response remained on VP1 across different HLA-DR haplotypes (Fig. 4). At the level of individual peptides, HLA stratification revealed that the immunodominant single peptides within the immunodominant pools differed according to the donors' HLA-DR haplotype. This is not too surprising when considering that the peptide binding grooves of the main alleles show considerable differences with respect to their binding motifs. In this context it has to be mentioned that we did not formally establish HLA-restricted peptide recognition, which needs to be done with T cell lines or -clones and antigen-presenting cells, e.g. HLA-DR transfectants, matched for each individual HLA-DR molecule. Such a detailed analysis is not possible at the level of the entire JCV proteome and across several HLA-DR haplotypes, however, our data should provide a basis for future studies in this direction.

Next we examined the question if JCV-specific cellular immune responses are comparable to VLP seropositivity or if there are substantial differences. Since there is at present no commonly established definition for what constitutes a positive T cell response to JCV and hence indicative if the individual has been exposed to the virus, we arbitrarily defined thresholds for the cellular immune response against JCV peptides or JCV VLP, for urinary viral shedding, and for the humoral immune response. To determine if an individual shows a positive JCV-specific T cell response we took into

account the fraction of JCV peptide pool-reactive wells. We found positive cellular response to JCV in 28.6 - 77.6% depending on the threshold. Other ways to determine the status of exposure to or infection with JCV are urinary excretion and the presence of JCV-specific antibodies. Here we found a positivity ranging from 36.4 - 45.5% (urinary excretion) and 42.6 - 89.4% (anti-VP1 antibody response), depending on the threshold and criterion. Since only approximately 50% of JCV-exposed individuals shed virus in the urine, ascertainment of JCV exposure cannot be based on JCV urinary excretion alone. On the other hand, an individual, who sheds virus in the urine, must be JCV infected even if JCV-specific T cell- or antibody responses are low or borderline. Based on our thresholds for positive T cell- and antibody responses, we assume that JCV exposure in our cohort ranged between between 60 and 80%. However, to formally address this issue, much larger cohorts need to be examined and compared systematically.

The protein triggering the highest overall proliferation is the major capsid protein VP1 and the amino-terminal end of the minor capsid protein VP2 (Fig. 3A, B). However, most donors also recognized other JCV proteins (Fig. S3), and the preference for a JCV protein varied not only among individual donors, but also among groups that had been stratified according to HLA-class II haplotype (Fig. S2 and Fig. S3).

As already mentioned above, when assessing immunodominance we considered the strength of the proliferative responses and the precursor frequency of antigen-specific T cells. The latter measure expresses the fraction of T cells of an individual that is specific for JCV, while the prevalence of immunity in a population provides information as to what fraction of a population responds to a specific antigen. All three measures provide distinct information and can be used to determine immunodominance. Only peptide pools fulfilling all three criteria for immunodominance were considered here and the peptides within these pools were investigated individually. With this strategy, we found peptides 41 (VP1 (123-137), SNGQASHDNGAGKPV) and 82 (VP2 (39-51), EVEIASLATVEGI) causing strong proliferation in the entire cohort of HD. Peptides 81 and 82, as well as peptides 154 and 155 overlap by 5aa, which may in part explain the proliferation to both. It is possible that the optimal and most stimulatory peptide is situated between peptide 81 and 82, or

623 between other peptides, that were not identified here, due to the overlap of only 5aa.
624 However, the broad specificity of the $CD4^+$ T cell responses indicates that multiple JCV
625 peptides can be recognized by Caucasian individuals, and this is consistent with the fact
626 that JCV is a highly prevalent virus, to which the human immune system is well
627 adapted.

628 Previously, two $CD8^+$ T cell epitopes, peptides VP1 (100-109) and VP1 (36-44),
629 have been described as immunodominant in HLA-A*0201-restricted donors (12, 31). In
630 our study, the VP1 (100-109) peptide (ILMWEAVTL) is represented by peptides 31
631 (VP1 (91-105); LNEDLTCGN*ILMWEA*) and peptide 32 (VP1 (101-115);
632 *LMWEAVTL*KTEVIGV) in peptide pool 6. The VP1 (36-44) peptide (SITEVECFL) is
633 covered by the peptide 20 (VP1 (34-48); VD*SITEVECFL*TPEM) in pool 4. Both pools 4
634 and 6 were among the 12 and 6 pools respectively that elicited the strongest responses
635 (Fig. 3B), and $DR15^+$ and $DR17^+$ individuals recognized pool 6 as the second strongest
636 and strongest pool, respectively (Fig. 4B). Further support for the relevance of peptide
637 VP1 (36-44) and peptide 20 from pool 4 (together with only 7 other JCV peptides)
638 comes from our recent data that these peptides are relevant targets for brain-infiltrating
639 $CD4^+$ T cells that had been isolated from a brain biopsy of a patient suffering from PML-
640 immune reconstitution inflammatory syndrome (3). We also demonstrated $CD8^+$ T cells
641 recognizing JCV VP1 (36-44) in the brain biopsy of this $HLA\text{-}A2^+$ MS patient, although
642 the $CD4^+$ T cells were more prevalent in the biopsy than $CD8^+$ T cells. Data from the
643 latter study and work presented here indicate that $CD8^+$ T cells and $CD4^+$ T cells
644 recognize related epitopes and that the JCV peptide VP1 (36-44) is of particular
645 relevance.

646 Regarding the influence of certain HLA-DR haplotypes on cellular immune
647 responsiveness against JCV, the comparably very low or absent reactivity in
648 $DRB1*04:01^+$ individuals was of particular interest. Since all individuals in the two
649 $DRB1*04^+$ subgroups were heterozygote for DRB1*04:01 or DRB1*04:other except for
650 one, and the effect of low proliferative response was not more pronounced in the
651 homozygote $DRB1*04:01^+$ individual, we conclude that the DRB1*04:01 haplotype has
652 a dominant-negative influence on the JCV-specific proliferative response. It remains to
653 be elucidated, which mechanisms lead to this gross reduction of JCV-specific T cell

responses in heterozygote DRB1*04:01$^+$ HD. Several associations between HLA-DRB1*04:01 and specific infections have been described in epidemiological studies, in which a considerable number of heterozygote donors was included: HLA-DRB1*04:01 was reported to be associated with slower progression of HIV-1 infection (32), clearance of chronic hepatitis C virus infection (38), and low hepatitis C virus activity (69). Furthermore, DRB1*04:01 is associated with increased risk for RA (45), mixed connective tissue disease (11) and insulin-dependent diabetes mellitus (57), as well as with a reduced age at onset of multiple sclerosis (MS) (68). DRB1*04:01$^+$ MS patients show a restricted T cell receptor repertoire in the context of the T cell response to one immunodominant myelin basic protein (residues 111-129) peptide (43). Taken together, these reports appear to document dominant-positive effects of the HLA-DRB1*04:01 allele. However, no experimentally proven domination of the DRB1*04:01 allele leading to reduced or abrogated function of other HLA-DR alleles has been reported. Previously, an HLA-associated low responsiveness to streptococcal antigen in healthy donors has been described and linked to the DR2-DQA1*0102-DQB1*0602(DQw6)-Dw2 haplotype and DR2-DQA1*0103-DQB1*0601 (DQw6)-Dw12 haplotype (28, 46). One possible mechanism for such low responsiveness inherited in a dominant fashion is clonal deletion. A second mechanism could involve the deletion of JCV-specific T cells by chronic antigenic restimulation *in situ*. A third possibility is the induction of a T regulatory phenotype of JCV-specific T cells responding to HLA-DRB1*04:01$^+$ antigen presenting cells. Whether the HLA-DRB1*04:01 allele itself or a immunosuppressive gene in strong linkage disequilibrium confers for this effect is of further interest, but remains to be clarified.

Additionally, we observed a B cell-skewed JCV-specific immune response in DRB1*04:01$^+$ individuals. The mechanisms underlying a strong humoral anti-JCV response in HLA-DRB1*04:01-restricted donors and at the same time low to absent CD4$^+$ T cell response remain to be elucidated. It could represent a T cell-independent JCV-specific B cell response with the second stimulation signal delivered by the JCV antigen itself, e.g. binding to Toll-like receptors or extensive cross-linking of the membrane. Such a T cell-independent virus-specific antibody response has been shown in mice lacking T cells and infected with mouse-PyV (56). A second mechanism

685 could involve a cross-reactive B-cell response, which recognizes not only JCV-encoded
686 epitopes, but also similar epitopes encoded by other human polyomaviruses or
687 infectious agents, which has been generated by $CD4^+$ T cells specific against other
688 human polyomaviruses or infectious agents, but not JCV. This mechanism could
689 operate in the absence of JCV-specific $CD4^+$ T cell responses and lead to JCV-specific
690 B cell responses that are only detectable due to cross-reactivity between epitopes of
691 JCV and other human polyomaviruses or other infectious agents.

692 Antibody cross-reactivity has been suggested between JCV and BKV VP1
693 proteins (23), BKV and S40 VP1 proteins (29, 64), JCV and SV40 VP1 proteins (29,
694 64). However, little or no antibody cross-reactivity between BKV and JCV VP1 proteins
695 or between SV40 and both JCV and BKV VP1 proteins has been reported in the
696 majority of studies (4, 15, 25, 36, 54, 55, 63, 64), and no antibody cross-reactivity was
697 found between VP1 proteins of other polyomaviruses, e.g. Lymphotropic Polyomavirus
698 (LPV) versus MCV or KIV versus WUV (29). Since we also did not find serum cross-
699 reactivity between JCV VP1 and its most related VP1 protein among human
700 polyomaviruses (BKV VP1) in a representative set of sera from HLA-DRB1*04:01^+ as
701 well as from HLA-DRB1*04:01^- donors, we expect the latter scenario to be less likely.

702 A third mechanism could be that the JCV-specific B cell response and a long-
703 lasting JCV-specific B cell memory had been generated with $CD4^+$ T cell help earlier in
704 life and that JCV-specific T cells had been deleted by chronic antigenic restimulation *in*
705 *situ*. It is well known that the so-called Type 2 helper T cells (T_h2) subset of $CD4^+$ T cells
706 activate B cells to provide neutralizing antibody production of various IgG isotypes by
707 $CD4^+$ T cell mediated production of interleukin (IL)-4 and IL-5 (42), whereas type 1
708 helper T cells (T_h1) cells producing IL-2 and interferon-γ (IFN-γ) favor clonal expansion
709 of cytotoxic $CD8^+$ T cells and macrophage activation. There are only few reports on an
710 influence of MHC class II genes in shifting the $CD4^+$ T cell phenotype in humans and
711 thereby predetermining an immune response, which is skewed to be more cell- or more
712 antibody-mediated. Agrewala and Wilkinson (1) showed that the p(91-110) epitope of
713 the 16-kDa antigen of *M. tuberculosis* is able to elicit a T_h1 or T_h2 response depending
714 on the HLA-DR haplotype of the donor. It was suggested, that a high MHC-peptide
715 affinity leads to a T_h1 response and lower binding affinities to a T_h2 response (33, 44).

716 Wen et al. (67) showed in HLA-DR4 transgenic mice that DRB1*04:01-encoded
717 molecules may promote a Th2 immune response. Furthermore, HLA-DRB1*04:01 has
718 been associated with the development of antibodies against interferon-beta in MS
719 patients and autoantibodies against insulin (9, 26).

720 The above previously described associations between higher or lower prevalence
721 of a specific infection or variations in disease presentation as well as the association
722 with certain autoimmune diseases are poorly understood at the functional level. They
723 argue, however, that specific host-pathogen interactions may alter immune
724 responsiveness in a way that impacts on the outcome of the infection. The fact that
725 such associations have been observed even for much larger/complex organisms than
726 JCV, e.g. M. *tuberculosis*, argue that the reasons must be more complex than lack of
727 binding of peptides to the DRB1*04:01 allele. The molecular/cellular mechanisms
728 governing a low/absent $CD4^+$ T cell response to JCV in DRB1*04:01^+ individuals
729 together with a very strong humoral response and lack of urinary shedding, its
730 implication for JCV immune control clearly merit further study.

731 Secondary to HLA DRB1*04:01, also DRB1*01^+ individuals demonstrated low
732 proliferation on JCV peptide pools, but less pronounced than in the DRB1*04:01
733 subgroup (Fig. 4C). Additionally, PBMC from DRB1*01^+ HD responded less to TTxd
734 stimulation than other DR groups. However, these differences were not significant.
735 Related to viral immunity, HLA-DRB1*01 has been associated with resistance to HIV
736 infection (34), with clearance of HCV infection (16, 39, 59) and with a good
737 responsiveness to HBsAG vaccination (10). More importantly, in analogy to HLA-DRB1-
738 04:01, HLA-DRB1*01:01 is associated with increased risk for RA (45), and these
739 commonalities with DRB1*04:01 and other RA-associated DR alleles have been
740 attributed to a shared motif in the peptide binding grooves of these DR molecules.
741 Whether reduced JCV-specific T cell responses in HLA-DRB1-04:01^+ and HLA-
742 DRB1*01:01^+ individuals with RA contribute to the precipitation of PML, especially
743 during immunomodulatory treatment such as rituximab (7), remains to be elucidated in
744 larger populations. In comparison to RA patients, PML occurs even more frequently in
745 patients with systemic lupus erythematosus (40) and natalizumab-treated MS patients

746 (35, 58, 61). Whether HLA associations contribute to an elevated PML risk needs to be
747 clarified, however, in larger population-based studies.

748 Our assay does not allow us to distinguish directly between $CD4^+$ T cell and
749 $CD8^+$ T cell driven proliferation. We have chosen 15mer peptides, which preferentially
750 stimulate $CD4^+$ T cell proliferation, since this length can be accommodated by the open
751 HLA-class II binding groove in contrast to the HLA-class I binding pocket, which is
752 closed and optimally accommodates peptides of 8-10 amino acids length. In order to
753 use the most sensitive assay with a broad dynamic range that allows us to detect even
754 small numbers of proliferating cells reliably we used thymidine incorporation as readout.
755 Alternative approaches using CFSE or enzyme-linked immunosorbent spots (ELISPOT)
756 are less sensitive, but the former can distinguish between $CD4^+$ or $CD8^+$ T cell
757 proliferation. When checking the composition of the proliferating T cell population after
758 JCV peptide- or protein stimulation by flow cytometry, we observed mainly $CD4^+$ T cells
759 supporting the above assumption. This does not verify exclusive $CD4^+$ T cell
760 proliferation, but that strong immunogenicity of some of the peptides mainly stems from
761 $CD4^+$ T cells with the possibility that $CD8^+$ T cells contribute as well. By choosing an
762 overlap of only 5 aa between adjacent peptides, we may have missed potential epitopes
763 not covered by our selection of peptides. However, a wide range of different epitopes
764 scattered over the entire JCV proteome may be recognized by single healthy donors as
765 shown in Fig. 1 and 3, peptide pools from all JCV proteins were recognized (Fig. S2)
766 and there was high inter-individual and inter-HLA-DR-group variability regarding
767 preference and strength of reactivity against single JCV peptide pools (Fig. S3),
768 indicating that a set of 15-mer peptides overlapping by 5 aa and covering the whole JCV
769 proteome is sufficient to uncover the intra- and interindividual variability in epitope
770 specificity and immunodominant JCV $CD4^+$ T cell epitopes.

771 We are aware that our data give rise to a number of questions. They do,
772 however, provide a good basis for future, more focused studies on specific aspects as
773 well as examination of larger patient numbers, which may employ additional tools
774 including HLA-class II tetramers loaded with JCV peptides and subsequent screening of
775 populations of interest, like MS patients receiving natalizumab and other populations at
776 risk for PML. In our view, assessing the T cell reactivity will, besides serological anti-

JCV responses and JCV viral DNA testing, provide important additional information with respect to virus exposure of the individual and the robustness of her/his immune status. Studying the strength, antigen fine specificity and in the future also functional phenotype of the T cell response against JCV is expected to be useful as a basis for subsequent studies on JCV immune control, for diagnostic purposes and also for the development of anti JCV vaccination.

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Figure Legends

Figure 1 JCV genome organization, protein sequence variability and mapping of peptide pools. (A) The genomic organization of the JCV reference genome (NC_001699), consisting of 5130 bp, is shown with the nucleotide positions of all open reading frames indicated by colored arrows. Nucleotides are numbered relative to the JCV reference genome (NC_001699). Large T antigen (LTAg, dark blue arrow), small T antigen (STAg, light blue arrow), three additional alternative splice variants of the large T antigen (T'135, T'136 and T'165, dark blue arrows), agnoprotein (agno, red arrow), VP1 (dark green arrow), VP2 (light green arrow) and VP3 (orange arrow) are shown, as well as early and late mRNAs (grey, dotted arrows), the tandem repeats (TR, grey boxes) in the non-coding region (NCR, grey line) and JCV miRNA (miR-J1, black dot). Introns within the respective open reading frame are depicted as boxes with dashed lines. (B) Sequence variability of amino acid sequences among different JCV strains (continuous line) and among nine human PyVs (dashed line) comprised of BKV (accession number NC_001538), JCV (NC_001699), KIV (NC_009238), WUV (NC_009539), MCV (NC_010277), HPyV6 (NC_014406), HPyV7 (NC_014407), TSV (NC_014361) and HPyV9 (NC_015150). Similarity scores were plotted against the respective amino acid of each protein and matched to the relative nucleotide position of the JCV reference genome (NC_001699). (C) Mapping of 42 peptide pools (colored boxes) to the relative nucleotide position of the JCV reference genome (NC_001699). LTAg, STAg, T'135, T'136 and T'165, agno, VP1, VP2 and VP3 (grey-shaded boxes) are shown, as well as early and late mRNAs (black lines) including introns (dashed lines) and polyadenylated 3'-ends (3' (A)n), in relation the JCV reference genome (NC_001699). Each pool consists of 5 different peptides. 169 peptides overlap by 5 amino acids (pep; non-hatched boxes) and 35 peptides contain single amino acid substitutions derived from common JCV variants (var; hatched boxes). Agno is covered by 3 peptide pools (pools 1-3, red), where pool 3 contains variants of peptides 5 (pool 1) and 7, 9 and 12 (pool 2), all matching to agno. VP1 is covered by 13 peptide pools (pools 4-16, dark green), where pools 12 to 16 contain variants of peptides included in VP1 pools 4 to 11. VP2 is covered by 8 peptide pools (pools 17-24, light green), where

pool 24 contain variants of peptides 96 (pool 20) and 106 (pool 22). VP3, the sequence of which is identical to the N-terminal part of VP2 is covered by 6 peptide pools (peptides 90-92 from pool 19 and pools 20-24, orange), where pool 24 contains variants of peptides 96 (pool 20) and 106 (pool 22). LTAg is covered by 16 peptide pools (pools 25-40, dark blue) and STAg is covered by 5 peptide pools (pools 25, 26 and 40-42, light blue), where pool 40 contains variants of peptides 185, 187 and 189 included in pool 39 (LTAg) and variants of peptides 193 and 195 includes in pool 41 (STAg).

FIG 2 Inter-individual variability in JCV-specific immune response in healthy donors. (A, left graphs) Proliferation of PBMC from three representative HD to 42 JCV-derived peptide pools as measured by ^{3}H thymidine incorporation. PBMC were stimulated with JCV peptides assembled in 42 peptide pools (x-axis) and eight wells per pool. Pools containing peptide variants are marked (v). Pool 3 contains variants of peptides included in pool 1 and 2 (agno). Pools 12 to 16 contain variants of peptides included in pools 4 to 11 (VP1). Pool 40 contains variants of peptides included in pool 39 (LTAg) and 41 (STAg). Dots represent single stimulation indices (SI) (y-axis) and were considered positive when SI ≥ 3 (horizontal line). (A, right graphs) Histograms representing the mean SI of unstimulated and TTxd-stimulated wells. (B) A representative sample shows cell proliferation by ^{3}H thymidine incorporation (left graph; mean SI [^{3}H-thymidine] ± standard deviation (SD); one dot corresponds to 2 x 10^{5} stimulated PBMC) and preferential proliferation and activation of $CD4^{+}$ T cells over $CD8^{+}$ T cells after stimulation by JCV peptide pools 7 and 10 as measured by CFSE dilution (central graph; mean SI [CFSE] ± SD; one dot corresponds to 10^{6} stimulated PBMC) and intracellular cytokine (IFN-γ) staining (right graph; mean SI [IFN-γ] ± SD; one dot corresponds to 10^{6} stimulated PBMC) in a FACS analysis. Peptide pools 7 and 10 were selected, because HD29 showed pool 7- and pool 10-reactive wells as measured by ^{3}H thymidine incorporation. (C) Spectrum of anti-JCV humoral responses measured by ELISA using normalized optical density at 450nm (nOD_{450}) in Ficoll supernatants of cohort 1 (left graph) and sera of cohort 2 (right graph). Ficoll supernatants were diluted by the factor 1.2 in relation to sera. (D) Urinary JC viral shedding expressed as $\log_{10}$ GC/ml.

FIG 3 Proliferation of PBMC to peptide pools and individual peptides. (A) Immunogenicity of 42 pools was assessed with regard to stimulatory capacity (Sum of SI>3) (upper graph), estimated precursor frequency (percentage of positive wells) (middle graph) and prevalence in the tested cohort 1 (percentage of positive donors) (lower graph). (B) Graph showing the reactivity score (RS) of the same cohort corresponding to each pool (Σ(SI>3) x percentage of reactive wells x percentage ofreactive individuals), which accounts for all three aspects of immunogenicity. Pools containing peptide variants are marked (v). Pool 3 contains variants of peptides included in pool 1 and 2 (agno). Pools 12 to 16 contain variants of peptides included in pools 4 to 11 (VP1). Pool 40 contains variants of peptides included in pool 39 (LTAg) and 41 (STAg). Pools 14, 17, 32, and 33 are most immunogenic in all three aspects (blue, green, yellow, red). (C) Graphs showing the RS of cohort 2 corresponding to each of the 20 single peptides from pools 14, 17, 32 and 33. The higher proliferations were evoked by peptide 41 [VP1 (123-137) V3], 81 [VP2 (30-43)], 82 [VP2 (39-51)], and 162 [(LTAg (415-429)].

FIG 4 HLA-restricted proliferation to JCV peptide pools. (A) Frequency of DRB1 expression among 49 HD from cohort 1. (B) Proliferation measured as RS of PBMC from $DR1^+$, $DR4^+$, $DR7^+$, $DR11^+$, $DR13^+$, $DR15^+$ and $DR17^+$ HD to 42 peptide pools. Because most donors are heterozygote with respect to DRB1* alleles, the same donor might appear in two different HLA cohorts. For quantitative comparison of proliferation, RS values in each HLA group were normalized to a group of 10 HD. Pools containing peptide variants are marked (v). Pool 3 contains variants of peptides included in pool 1 and 2 (agno). Pools 12 to 16 contain variants of peptides included in pools 4 to 11 (VP1). Pool 40 contains variants of peptides included in pool 39 (LTAg) and 41 (STAg). (C) Proliferation of PBMC from $DR1^+$, $DR4^+$, $DR7^+$, $DR11^+$, $DR13^+$, $DR15^+$ and $DR17^+$ HD measured as sum of RS from all peptide pools. (D) Proliferation measured as RS of PBMC from $DR1^+$, $DR4^+$, $DR7^+$, $DR11^+$, $DR13^+$, $DR15^+$ and $DR17^+$ HD to TTxd. Values shown mean RS ± SEM.

FIG 5 HLA-restricted proliferation to individual JCV peptides. (A) Frequency of DRB1 expression among 20 HD from cohort 2. (B) Proliferation measured as RS of PBMC from $DR1^+$, $DR7^+$, $DR11^+$, $DR13^+$, $DR15^+$ and $DR17^+$ HD to JCV individual peptides, which were contained in the most immunogenic peptide pools (pools 14, 17, 32, and 33). Peptides contained in pool 14 (blue), peptides contained in pool 17 (green), peptides contained in pool 32 (yellow) and peptides contained in pool 33 (red). For quantitative comparison of proliferation, RS values in each HLA group were normalized to a group of 10 HD.

FIG 6 T cell proliferation to JCV, urinary viral shedding and JCV-specific antibody levels in DRB1*04:01^+ healthy donors. (A) Proliferation of PBMC from DRB1*04:01^+ HD (n=10) measured as RS to JCV peptide pools in comparison to PBMC from DRB1*04^+ other than DRB1*04:01^+ HD (n=5). For quantitative comparison of proliferation, RS values in each HLA group were normalized to a group of 10 HD. Pools containing peptide variants are marked (v). Pool 3 contains variants of peptides included in pool 1 and 2 (agno). Pools 12 to 16 contain variants of peptides included in pools 4 to 11 (VP1). Pool 40 contains variants of peptides included in pool 39 (LTAg) and 41 (STAg). (B) Proliferation of PBMC from DRB1*04:01^+ and DRB1*04^+ other than DRB1*04:01^+ HD measured as sum of RS from all peptide pools. (C) Proliferation measured as RS of PBMC from DRB1*04:01^+ and DRB1*04^+ other than DRB1*04:01^+ HD to TTxd. Values shown mean RS ± SEM. (D) JCV urinary viral load expressed as log10 GC/ml of DRB1*04:01^+ (n=7), DRB1*04^+ other than DRB1*04:01^+ (n=5) and non-$DR4^+$ HD (n=10). (E) VLP-specific antibody levels measured by ELISA using normalized optical density at 450nm (nOD_{450}) in sera from DRB1*04:01^+ HD (n=7), DRB1*04^+ HD other than DRB1*04:01^+ (n=5) and non-$DR4^+$ HD (n=10). (F) Antibody reactivity (mean nOD_{450} ± standard error of mean) to JCV VP1 proteins after competition with soluble BKV VP1 protein (BKV competition) or soluble JCV VP1 protein (JCV competition) in sera of DRB1*04:01^+ HD (n=5, left graph) and DRB1*04:01^- HD (n=7, right graph).

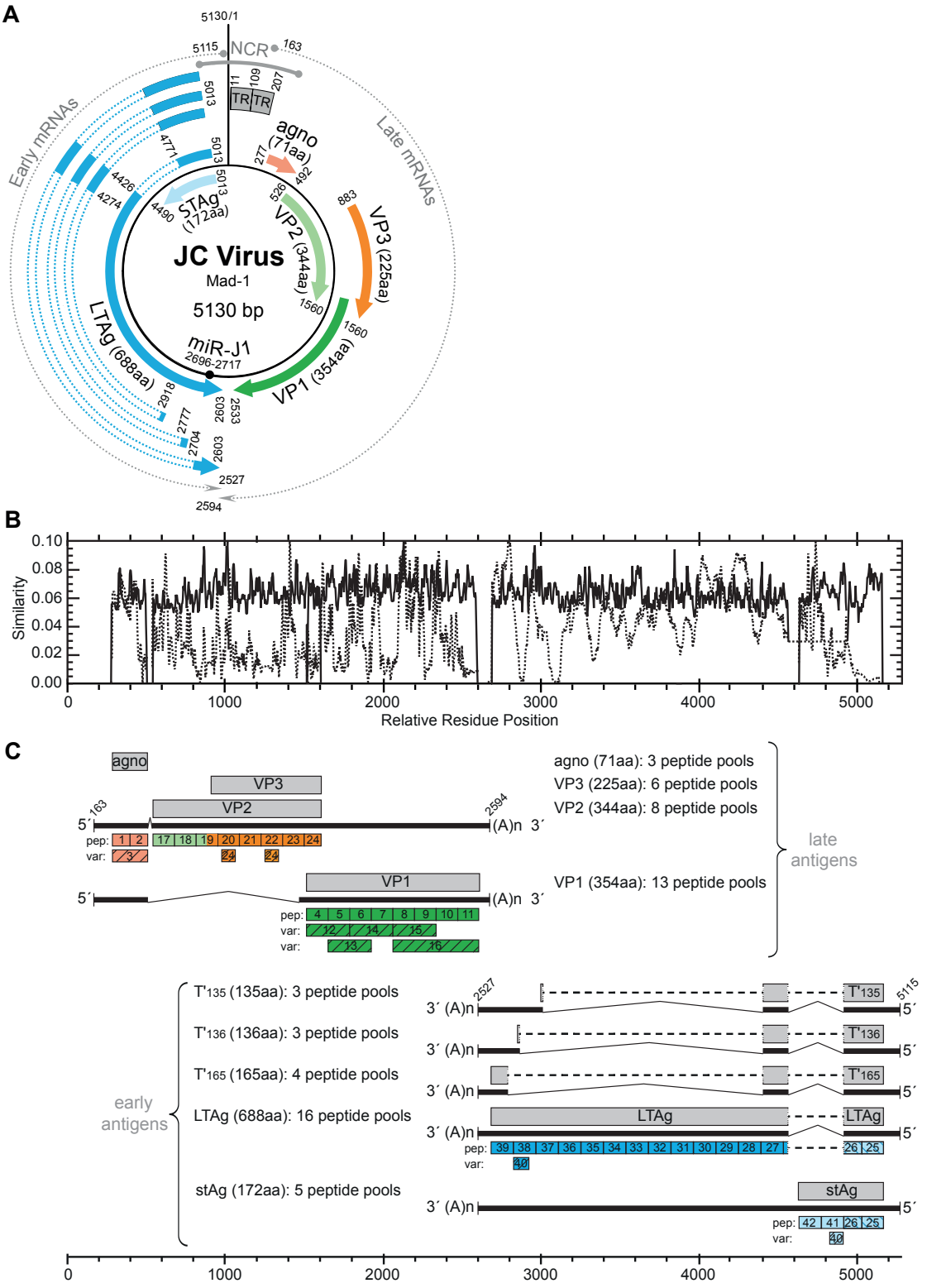

Fig 1 JCV genome organization, protein sequence variability and mapping of peptide pools.
(figure legend continued on the next page)

Figure 1 (figure legend continued)
(A) The genomic organization of the JCV reference genome (NC_001699), consisting of 5130 bp, is shown with the nucleotide positions of all open reading frames indicated by colored arrows. Nucleotides are numbered relative to the JCV reference genome (NC_001699). Large T antigen (LTAg, dark blue arrow), small T antigen (STAg, light blue arrow), three additional alternative splice variants of the large T antigen (T'135, T'136 and T'165, dark blue arrows), agnoprotein (agno, red arrow), VP1 (dark green arrow), VP2 (light green arrow) and VP3 (orange arrow) are shown, as well as early and late mRNAs (grey, dotted arrows), the tandem repeats (TR, grey boxes) in the non-coding region (NCR, grey line) and JCV miRNA (miR-J1, black dot). Introns within the respective open reading frame are depicted as boxes with dashed lines. (B) Sequence variability of amino acid sequences among different JCV strains (continuous line) and among nine human PyVs (dashed line) comprised of BKV (accession number NC_001538), JCV (NC_001699), KIV (NC_009238), WUV (NC_009539), MCV (NC_010277), HPyV6 (NC_014406), HPyV7 (NC_014407), TSV (NC_014361) and HPyV9 (NC_015150). Similarity scores were plotted against the respective amino acid of each protein and matched to the relative nucleotide position of the JCV reference genome (NC_001699). (C) Mapping of 42 peptide pools (colored boxes) to the relative nucleotide position of the JCV reference genome (NC_001699). LTAg, STAg, T'135, T'136 and T'165, agno, VP1, VP2 and VP3 (grey-shaded boxes) are shown, as well as early and late mRNAs (black lines) including introns (dashed lines) and polyadenylated 3'-ends (3' (A)n), in relation the JCV reference genome (NC_001699). Each pool consists of 5 different peptides. 169 peptides overlap by 5 amino acids (pep; non-hatched boxes) and 35 peptides contain single amino acid substitutions derived from common JCV variants (var; hatched boxes). Agno is covered by 3 peptide pools (pools 1-3, red), where pool 3 contains variants of peptides 5 (pool 1) and 7, 9 and 12 (pool 2), all matching to agno. VP1 is covered by 13 peptide pools (pools 4-16, dark green), where pools 12 to 16 contain variants of peptides included in VP1 pools 4 to 11. VP2 is covered by 8 peptide pools (pools 17-24, light green), where pool 24 contain variants of peptides 96 (pool 20) and 106 (pool 22). VP3, the sequence of which is identical to the N-terminal part of VP2 is covered by 6 peptide pools (peptides 90-92 from pool 19 and pools (peptides 90-92 from pool 19 and pools 20-24, orange), where pool 24 contains variants of peptides 96 (pool 20) and 106 (pool 22). LTAg is covered by 16 peptide pools (pools 25-40, dark blue) and STAg is covered by 5 peptide pools (pools 25, 26 and 40-42, light blue), where pool 40 contains variants of peptides 185, 187 and 189 included in pool 39 (LTAg) and variants of peptides 193 and 195 includes in pool 41 (STAg).

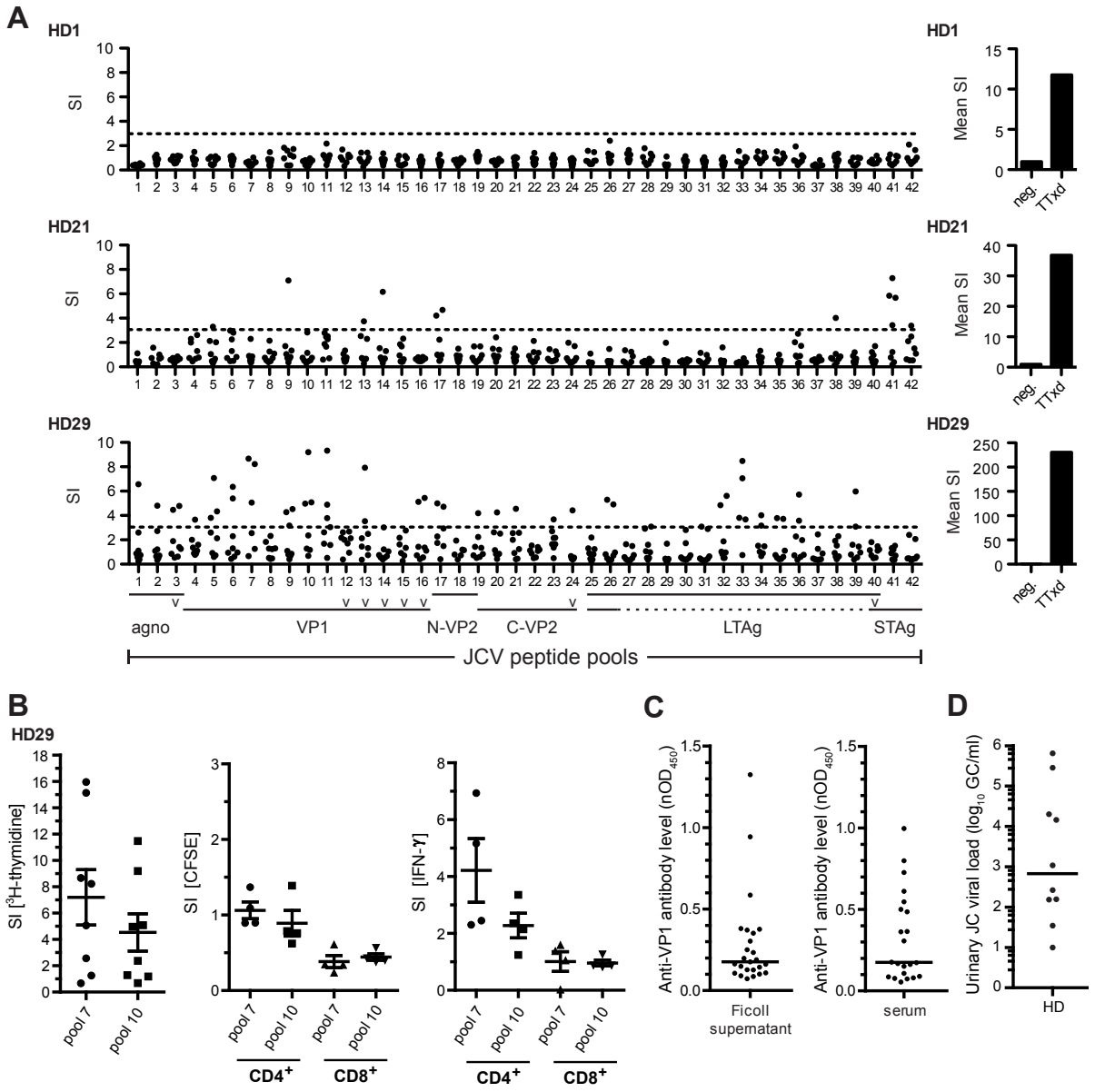

FIG 2 Inter-individual variability in JCV-specific immune response in healthy donors. (A, left graphs) Proliferation of PBMC from three representative HD to 42 JCV-derived peptide pools as measured by ^{3}H-thymidine incorporation. PBMC were stimulated with JCV peptides assembled in 42 peptide pools (x-axis) and eight wells per pool. Pools containing peptide variants are marked (v). Pool 3 contains variants of peptides included in pool 1 and 2 (agno). Pools 12 to 16 contain variants of peptides included in pools 4 to 11 (VP1). Pool 40 contains variants of peptides included in pool 39 (LTAg) and 41 (STAg). Dots represent single stimulation indices (SI) (y-axis) and were considered positive when SI ≥ 3 (horizontal line). (A, right graphs) Histograms representing the mean SI of unstimulated and TTxd-stimulated wells. (B) A representative sample shows cell proliferation by ^{3}H thymidine incorporation (left graph; mean SI [^{3}H-thymidine] ± standard deviation (SD); one dot corresponds to 2 x 10^{5} stimulated PBMC) and preferential proliferation and activation of CD4^{+} T cells over CD8^{+} T cells after stimulation by JCV peptide pools 7 and 10 as measured by CFSE dilution (central graph; mean SI [CFSE] ± SD; one dot corresponds to 10^{6} stimulated PBMC) and intracellular cytokine (IFN-γ) staining (right graph; mean SI [IFN-γ] ± SD; one dot corresponds to 10^{6} stimulated PBMC) in a FACS analysis. Peptide pools 7 and 10 were selected, because HD29 showed pool 7- and pool 10-reactive wells as measured by ^{3}H thymidine incorporation. (C) Spectrum of anti-JCV humoral responses measured by ELISA using normalized optical density at 450nm (nOD_{450}) in Ficoll supernatants of cohort 1 (left graph) and sera of cohort 2 (right graph). Ficoll supernatants were diluted by the factor 1.2 in relation to sera. (D) Urinary JC viral shedding expressed as $\log_{10}$ GC/ml.

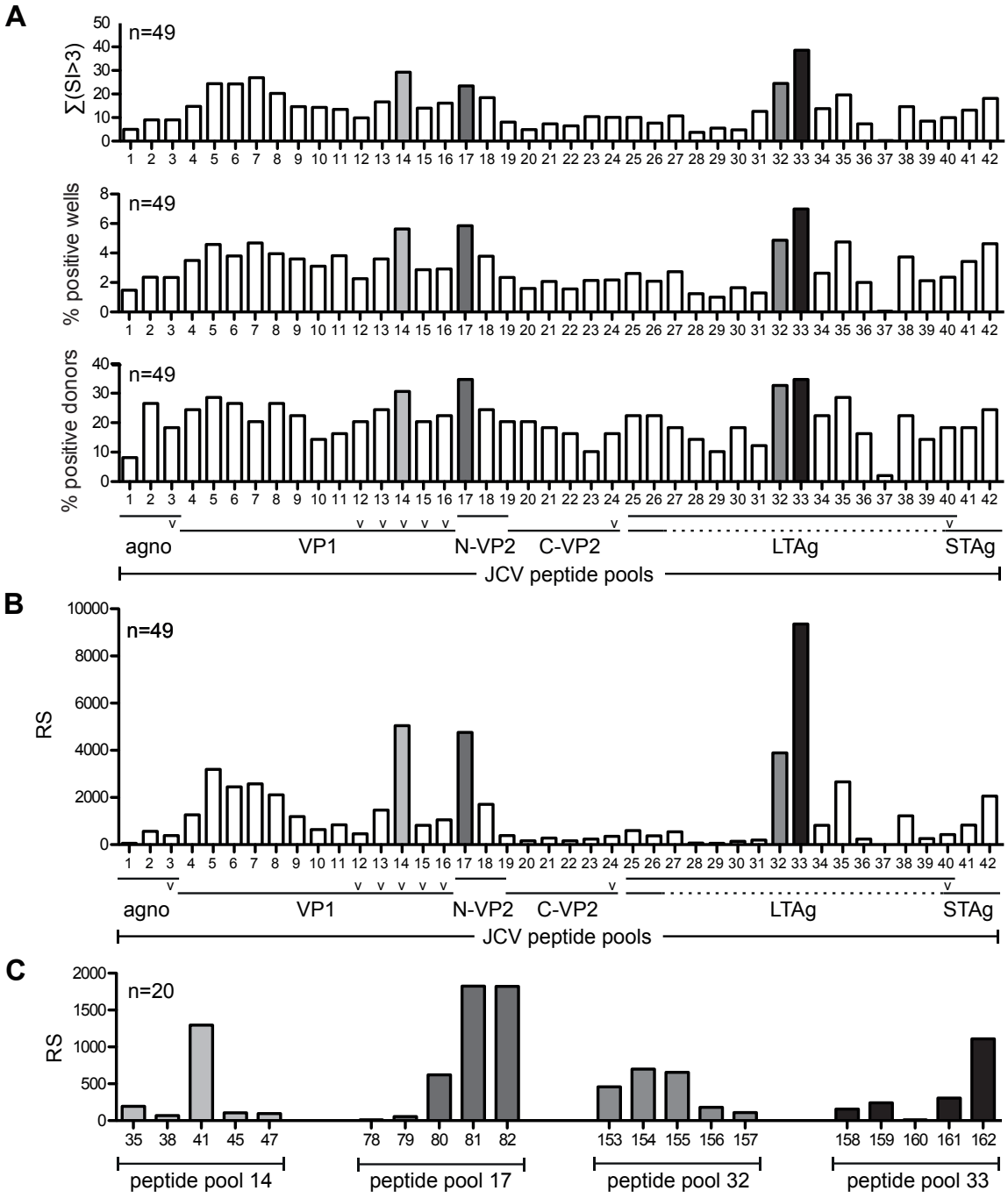

FIG 3 Proliferation of PBMC to peptide pools and individual peptides. (A) Immunogenicity of 42 pools was assessed with regard to stimulatory capacity (Sum of SI>3) (upper graph), estimated precursor frequency (percentage of positive wells) (middle graph) and prevalence in the tested cohort 1 (percentage of positive donors) (lower graph). (B) Graph showing the reactivity score (RS) of the same cohort corresponding to each pool (∑(SI>3) x percentage of reactive wells x percentage of reactive individuals), which accounts for all three aspects of immunogenicity. Pools containing peptide variants are marked (v). Pool 3 contains variants of peptides included in pool 1 and 2 (agno). Pools 12 to 16 contain variants of peptides included in pools 4 to 11 (VP1). Pool 40 contains variants of peptides included in pool 39 (LTAg) and 41 (STAg). Pools 14, 17, 32, and 33 are most immunogenic in all three aspects (blue, green, yellow, red). (C) Graphs showing the RS of cohort 2 corresponding to each of the 20 single peptides from pools 14, 17, 32 and 33. The higher proliferations were evoked by peptide 41 [VP1 (123-137) V3], 81 [VP2 (30-43)], 82 [VP2 (39-51)], and 162 [(LTAg (415-429)].

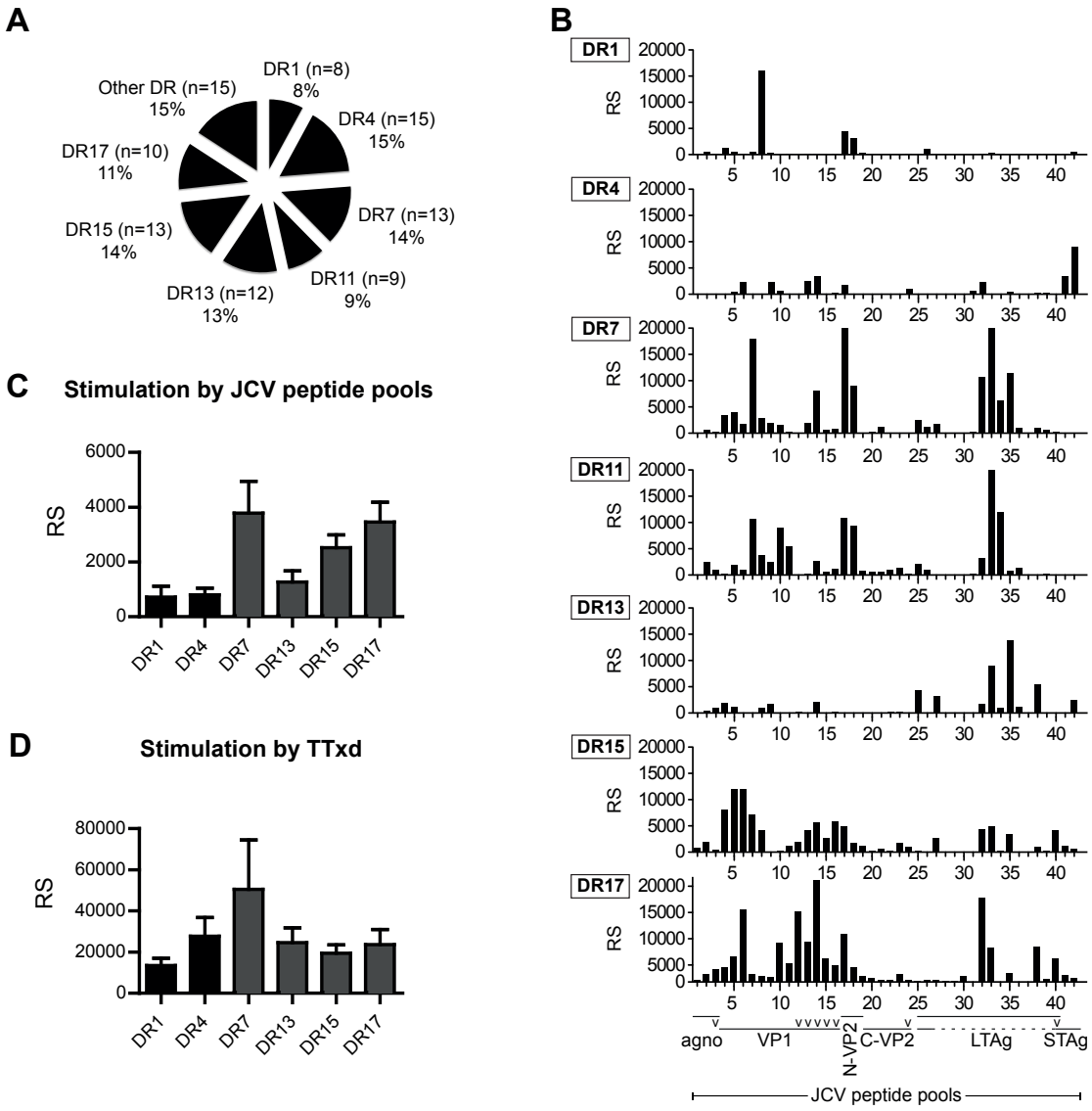

FIG 4 HLA-restricted proliferation to JCV peptide pools. (A) Frequency of DRB1 expression among 49 HD from cohort 1. (B) Proliferation measured as RS of PBMC from DR1$^+$, DR4$^+$, DR7$^+$, DR11$^+$, DR13$^+$, DR15$^+$ and DR17$^+$ HD to 42 peptide pools. Because most donors are heterozygote with respect to DRB1* alleles, the same donor might appear in two different HLA cohorts. For quantitative comparison of proliferation, RS values in each HLA group were normalized to a group of 10 HD. Pools containing peptide variants are marked (v). Pool 3 contains variants of peptides included in pool 1 and 2 (agno). Pools 12 to 16 contain variants of peptides included in pools 4 to 11 (VP1). Pool 40 contains variants of peptides included in pool 39 (LTAg) and 41 (STAg). (C) Proliferation of PBMC from DR1$^+$, DR4$^+$, DR7$^+$, DR11$^+$, DR13$^+$, DR15$^+$ and DR17$^+$ HD measured as sum of RS from all peptide pools. (D) Proliferation measured as RS of PBMC from DR1$^+$, DR4$^+$, DR7$^+$, DR11$^+$, DR13$^+$, DR15$^+$ and DR17$^+$ HD to TTxd. Values shown mean RS ± SEM.

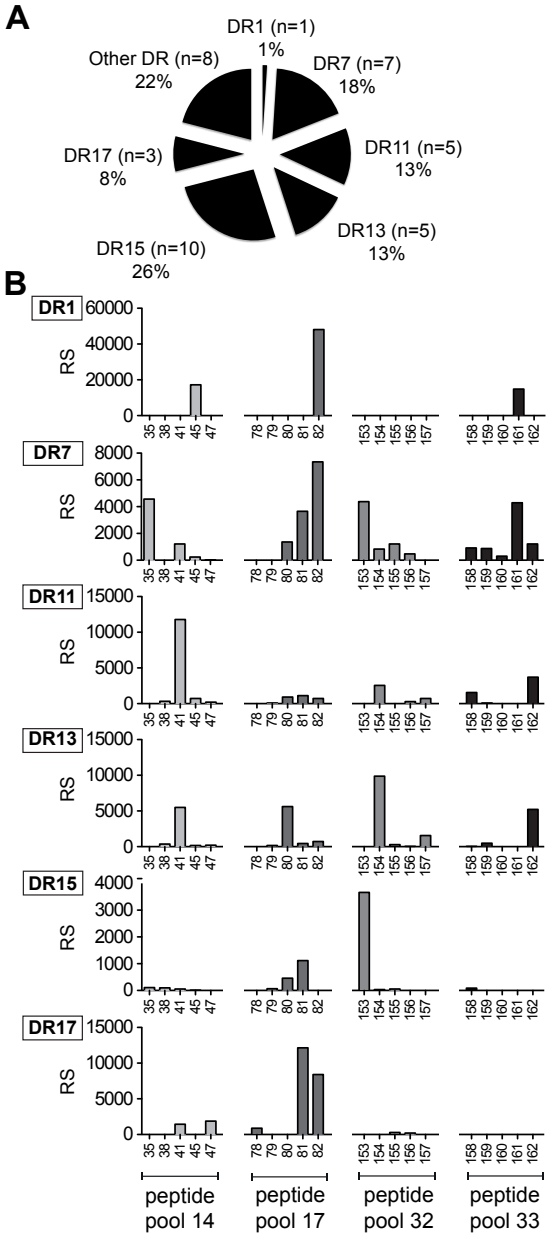

FIG 5 HLA-restricted proliferation to individual JCV peptides. (A) Frequency of DRB1 expression among 20 HD from cohort 2. (B) Proliferation measured as RS of PBMC from DR1$^+$, DR7$^+$, DR11$^+$, DR13$^+$, DR15$^+$ and DR17$^+$ HD to JCV individual peptides, which were contained in the most immunogenic peptide pools (pools 14, 17, 32, and 33). Peptides contained in pool 14 (blue), peptides contained in pool 17 (green), peptides contained in pool 32 (yellow) and peptides contained in pool 33 (red). For quantitative comparison of proliferation, RS values in each HLA group were normalized to a group of 10 HD.

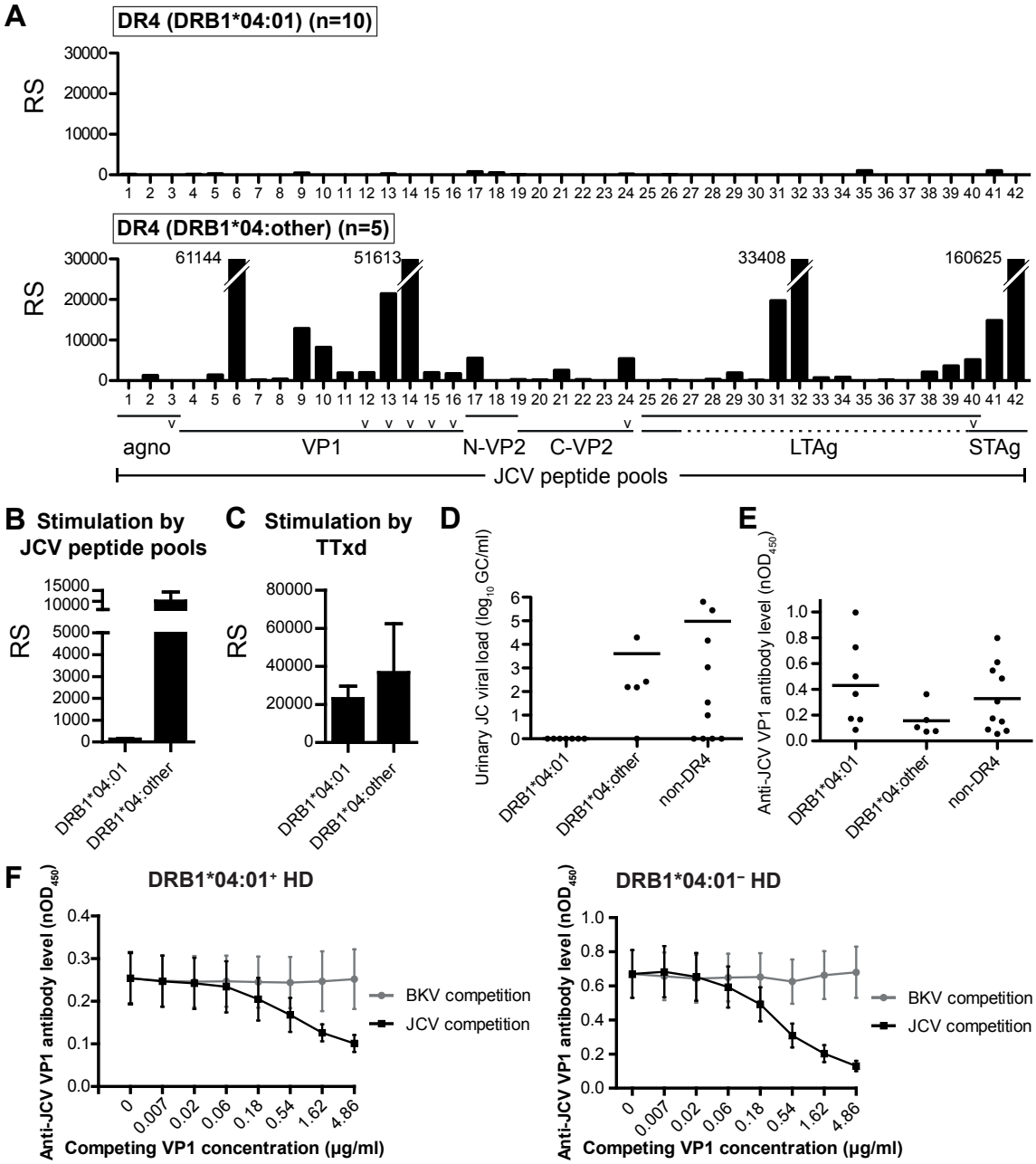

FIG 6 T cell proliferation to JCV, urinary viral shedding and JCV-specific antibody levels in DRB1*04:01+ healthy donors. (A) Proliferation of PBMC from DRB1*04:01$^+$ HD (n=10) measured as RS to JCV peptide pools in comparison to PBMC from DRB1*04$^+$ other than DRB1*04:01$^+$ HD (n=5). For quantitative comparison of proliferation, RS values in each HLA group were normalized to a group of 10 HD. Pools containing peptide variants are marked (v). Pool 3 contains variants of peptides included in pool 1 and 2 (agno). Pools 12 to 16 contain variants of peptides included in pools 4 to 11 (VP1). Pool 40 contains variants of peptides included in pool 39 (LTAg) and 41 (STAg). (B) Proliferation of PBMC from DRB1*04:01$^+$ and DRB1*04$^+$ other than DRB1*04:01$^+$ HD measured as sum of RS from all peptide pools. (C) Proliferation measured as RS of PBMC from DRB1*04:01$^+$ and DRB1*04$^+$ other than DRB1*04:01$^+$ HD to TTxd. Values shown mean RS ± SEM. (D) JCV urinary viral load expressed as $\log_{10}$ GC/ml of DRB1*04:01$^+$ (n=7), DRB1*04$^+$ other than DRB1*04:01+ (n=5) and non-DR4+ HD (n=10). (E) VLP-specific antibody levels measured by ELISA using normalized optical density at 450nm (nOD_{450}) in sera from DRB1*04:01$^+$ HD (n=7), DRB1*04$^+$ HD other than DRB1*04:01$^+$ (n=5) and non-DR4$^+$ HD (n=10). (F) Antibody reactivity (mean nOD_{450} ± standard error of mean) to JCV VP1 proteins after competition with soluble BKV VP1 protein (BKV competition) or soluble JCV VP1 protein (JCV competition) in sera of DRB1*04:01$^+$ HD (n=5, left graph) and DRB1*04:01$^-$ HD (n=7, right graph).

TABLE 1 Demographic data of HD and experiments performed in the study

	HD cohort 1 (n=49)	**HD cohort 2 (n=22)**
Gender (male:female)	1 : 1.33 (21 : 28)	1 : 2.14 (7 : 15)
Mean age at date of collection, years (range)	46.5 (23 – 72)	35.4 (26 – 46)
Caucasian, n	49	22
HLA-typed, n	49	22
Cellular response to peptide pools tested, n	49	0
Cellular response to single peptides and virus-like-particles tested, n	20	0
Humoral response tested, n	25	22
Urinary viral shedding tested, n	0	22

TABLE 2 Immunodominant JCV peptides and their maximum sequence identity with the proteome of eight other human PyVs[a]

Immunodominant JCV epitopes					Maximum identity with PyV proteome (%)	PyV protein and position	PyV peptide sequence
JCV peptide no.	JCV pool no.	JCV protein and position	JCV peptide sequence	Amino acids			
35	14	VP1 (108-122) V3	LKTEVIGVTALMNVH	15	66.7	TSV VP1 (119-133)	VKTEVVGVSSLVNVH
41	14	VP1 (123-137) V3	SNGQASHDNGAGKPV	15	46.7	MCV VP1 (131-145)	WDMKRVHDYGAGIPV
80	17	VP2 (20-34)	AATGFSVAEIAAGEA	15	100.0	BKV VP2 (20-34)	AATGFSVAEIAAGEA
81	17	VP2 (30-43)	AAGEAAATIEVEIA	14	85.7	BKV VP2 (30-43)	AAGEAAAAIEVQIA
82	17	VP2 (39-51)	EVEIASLATVEGI	13	92.3	BKV VP2 (39-51)	EVQIASLATVEGI
153	32	LTAg (328-342)	FADSKNQKSICQQAV	15	93.3	BKV LTAg (329-343)	FAESKNQKSICQQAV
154	32	LTAg (338-351)	CQQAVDTVAAKQRV	14	85.7	BKV LTAg (339-352)	CQQAVDTVLAKKRV
155	32	LTAg (347-361)	AKQRVDSIHMTREEM	15	80.0	BKV LTAg (348-362)	AKKRVDTLHMTREEM
161	33	LTAg (405-419)	VIYDFLKCIVLNIPK	15	73.3	BKV LTAg (406-420)	VIFDFLHCIVFNVPK
162	33	LTAg (415-429)	LNIPKKRYWLFKGPI	15	80.0	BKV LTAg (416-430)	FNVPKRRYWLFKGPI

[a] All protein sequences from the reference genomes of BKV (accession number NC_001538), KIV (NC_009238), WUV (NC_009539), MCV (NC_010277), HPyV6 (NC_014406), HPyV7 (NC_014407), TSV (NC_014361), HPyV9 (NC_015150) were used for epitope conservancy analysis (www.immuneepitope.org). Non-matching amino acids are grey-shaded and underlined.

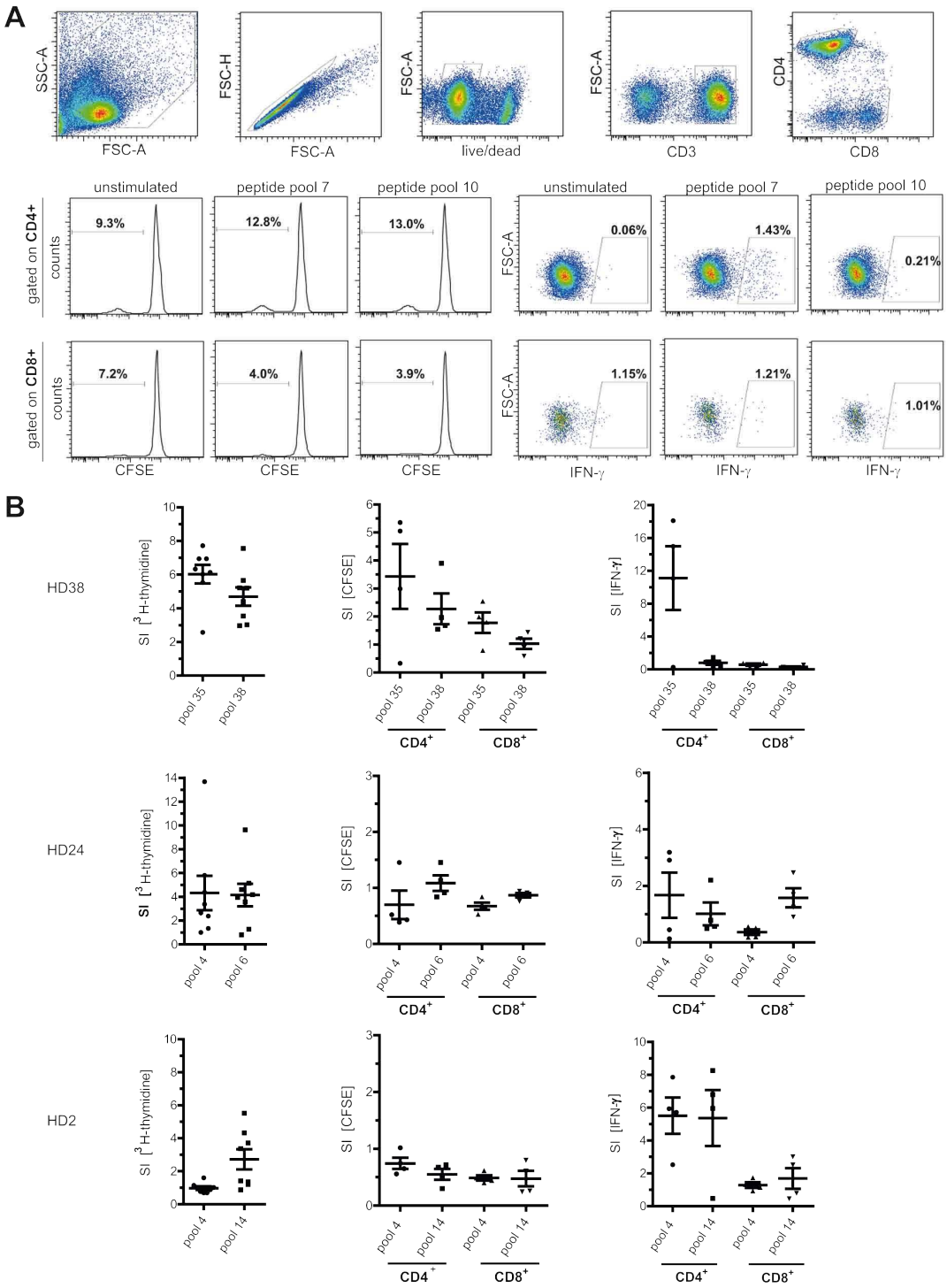

Figure S1 Gating strategy and stimulation indices for combined CFSE and IFN-γ ICS assays (figure legend continued on the next page).

Fig. S1 (figure legend continued)

A Gating on lymphocytes, singlet cells, live cells, $CD3^+$ cells, $CD4^+$ and $CD8^+$ cells allows for analysis of $CFSE^{dim}$ and IFN-γ^+ cells within $CD4^+$ and $CD8^+$ cells (HD 29).

B Three representative samples (HD38, HD24 and HD2 show cell proliferation by ^3H thymidine incorporation (left graph; mean SI [^3H-thymidine] ± standard deviation (SD); one dot corresponds to 2 x 10^5 stimulated PBMC) and preferential proliferation and activation of $CD4^+$ T cells over $CD8^+$ T cells as measured by CFSE dilution (central graph; mean SI [CFSE] ± SD; one dot corresponds to 10^6 stimulated PBMC) and intracellular cytokine (IFN-γ) staining (right graph; mean SI [IFN-γ] ± SD; one dot corresponds to 10^6 stimulated PBMC) in a FACS analysis after stimulation by JCV peptide pools identified as immunodominant in the respective donor by ^3H thymidine incorporation.

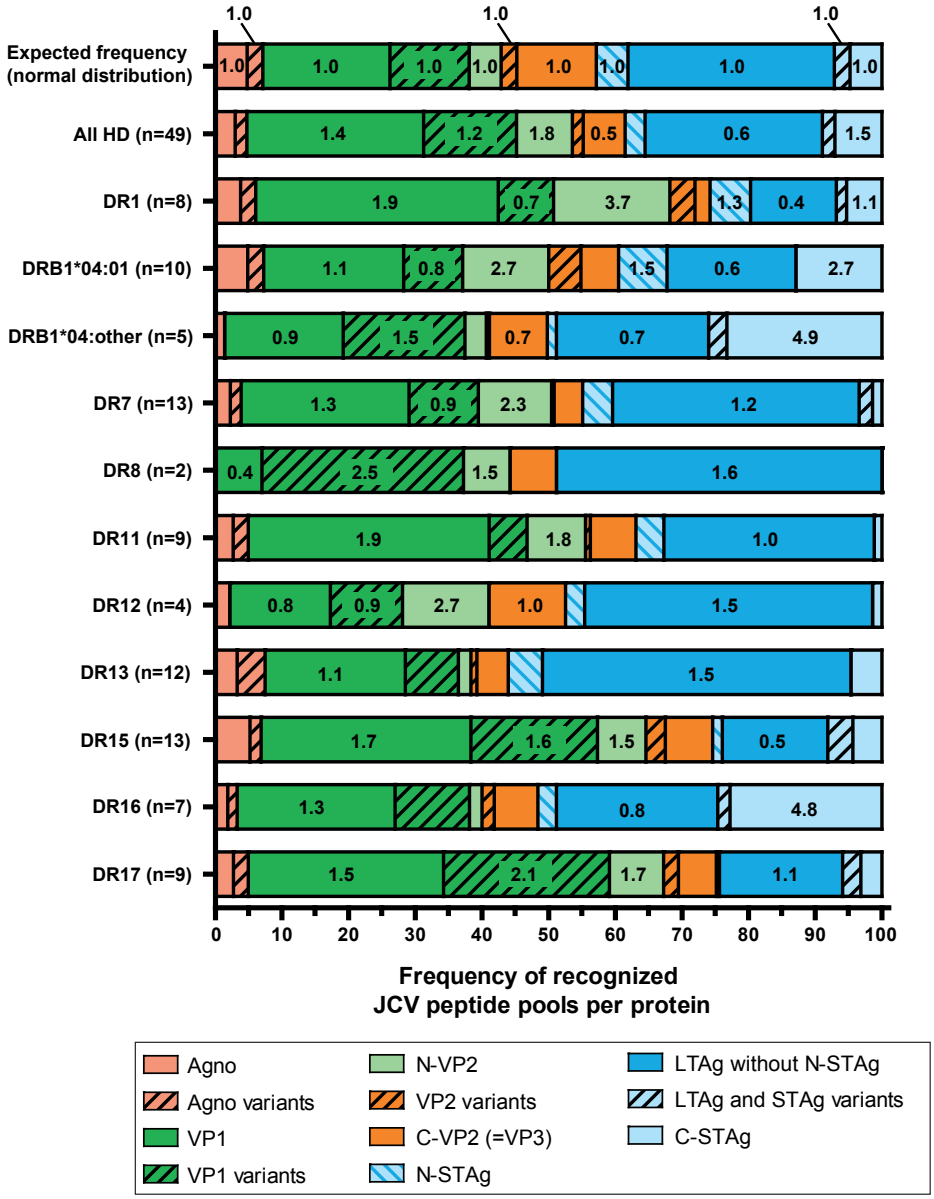

Fig. S2 Frequency distribution of JCV proteins recognized by T cells from healthy donors in accordance with HLA-DR alleles. T cell reactivity against JCV proteins in single HD was assessed by measuring the T cell response against 42 JCV-derived peptide pools, which contain 204 JCV-encoded peptides spanning the coding regions of all JCV proteins. Recognized peptide pools were assigned to the respective JCV protein. The bar shows the frequency distribution of JCV proteins among all positive peptide pools recognized by T cells of all HD (n=49) and after stratification of HD into HLA-DR groups (figure legend continued on the next page).

Fig. S2 (figure legend continued)

Agnoprotein (agno, red) and its variants containing common single amino acid mutations (agno variants, red shaded), VP1 (dark green) and its variants containing common single amino acid mutations (dark green shaded), aminoterminal VP2 (N-VP2, light green), VP3 corresponding to carboxyterminal VP2 (VP3 = C-VP2, orange) and its variants containing common single amino acid mutations (VP2 variants, orange shaded), aminoterminal small T antigen corresponding to the aminoterminal Large T antigen (N-STAg = N-LTAg, light blue shaded with dark blue), Large T antigen without aminoterminal LTAg (LTAg without N-STAg, dark blue), LTAg and STAg variants containing common single amino acid mutations (light blue shaded with black) and carboxyterminal STAg (C-STAg, light blue) are shown.

A ratio is calculated by dividing the real frequency of positive JCV peptide pools per protein by the virtual frequency expected at equal reactivity to all JCV peptide pools and is depicted within the bars. A number higher than 1 indicates that peptides derived from the respective protein have been recognized more often than expected. A number lower than 1 indicates that peptides derived from the respective protein have been recognized less often than expected.

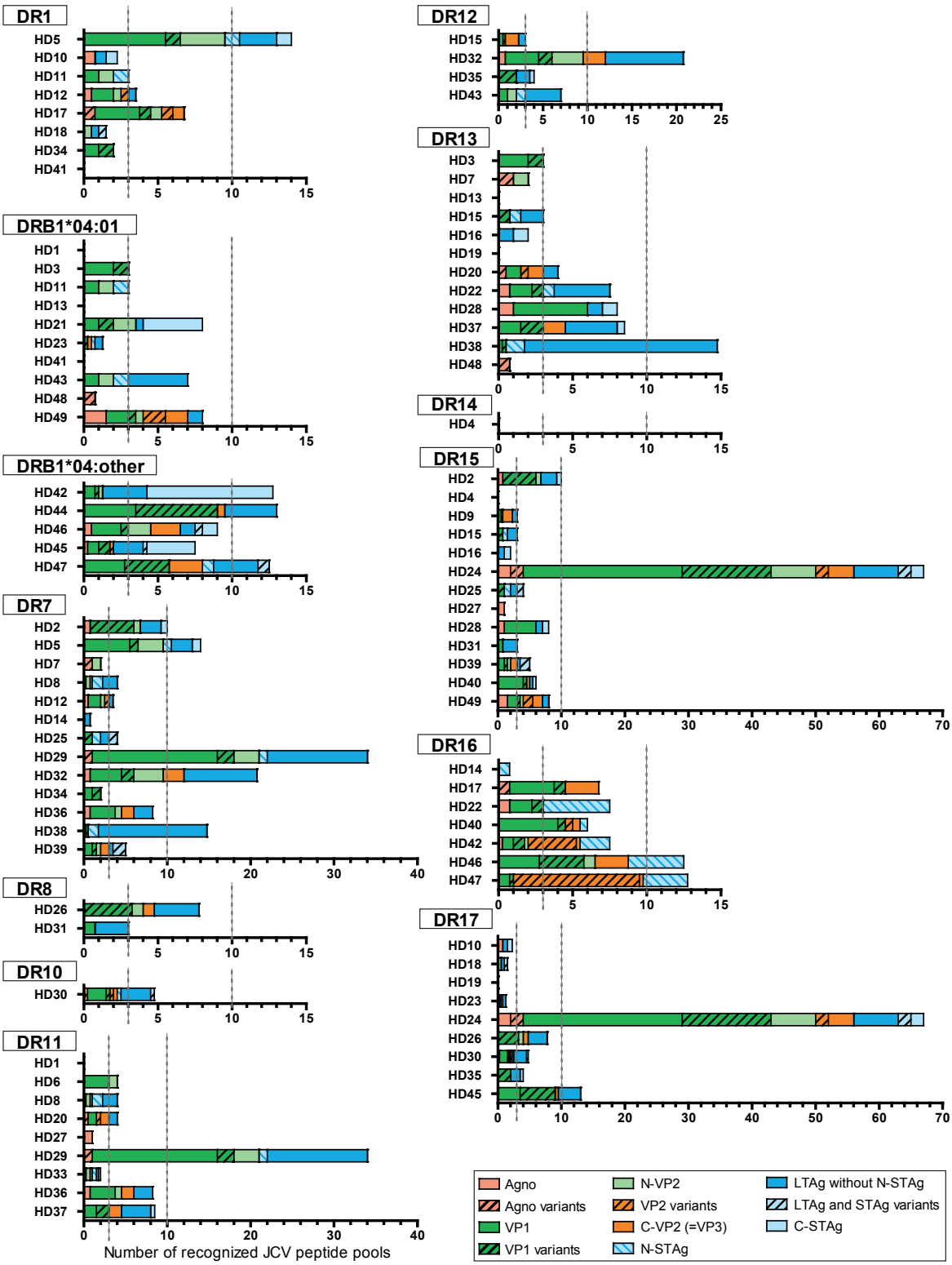

Figure S3 Single donor T cell reactivity against JCV proteins in HLA groups measured by peptide-specific responses (figure legend continued on the next page).

Figure S3 (figure legend continued)

Each bar represents the T cell response against JCV-derived peptide pools in a single HD, and the HD were grouped according to HLA-DR groups. The bar depicts the number of wells showing T cell reactivity against JCV peptide pools in each HD after seeding of 336 wells containing 8 repeats of 42 different peptide pools, which comprise of 204 different JCV-encoded peptides spanning the coding regions of all JCV proteins.

The recognized peptide pools are assigned to the six different JCV-encoded proteins. Agnoprotein (agno, red) and its variants containing common single amino acid mutations (agno variants, red shaded), VP1 (dark green) and its variants containing common single amino acid mutations (dark green shaded), aminoterminal VP2 (N-VP2, light green), VP3 corresponding to carboxyterminal VP2 (VP3 = C-VP2, orange) and its variants containing common single amino acid mutations (VP2 variants, orange shaded), aminoterminal small T antigen corresponding to the aminoterminal Large T antigen (N-STAg = N-LTAg, light blue shaded with dark blue), Large T antigen without aminoterminal LTAg (LTAg without N-STAg, dark blue), LTAg and STAg variants containing common single amino acid mutations (light blue shaded with black) and carboxyterminal STAg (C-STAg, light blue) are shown.

The number of positive peptide pools was corrected for background proliferation in each protein of each HD by calculating the $\sum(SI>3)$ for unstimulated wells (n=32), normalizing the value for the number of stimulated wells and subtracting it from the $\sum(SI>3)$ corresponding to each pool and donor. Two different thresholds for positivity were arbitrarily defined to identify HD with positive JCV-specific T cell responses, a low threshold considering that a HD has a positive JCV T cell response if she/he shows 3 or more wells with $SI \geq 3$, and a high threshold considering 10 or more wells with $SI \geq 10$ as a sign of positive JCV T cell response.

TABLE S1 HLA-DR and HLA-DQ alleles of all HD enrolled in cohort 1

Donor number	HLA serotype		HLA alleles							
	DR	DR	DRB1	DRB1	other DR	other DR	DQA1	DQA1	DQB1	DQB1
HD1	11	4	1101	0401	B3*0202	B4*01XX	03XX	05XX	0301	-
HD2	7	15	0701	1501	B4*01XX	B5*0101	0102	0201	0202	0602
HD3	13	4	1301	0401	B4*01XX	B3*0202	0102	03XX	0302	0603
HD4	15	14	1501	1404	B5*0101	B3*0202	0104	0102	0503	0602
HD5	1	7	0101	0701	B4*01XX	-	0101	0201	0202	0501
HD6	11	-	1101	-	B3*0202	-	05XX	-	0301	-
HD7	13	7	1301	0701	B4*01XX	B3*0101	0103	0201	0202	0603
HD8	11	7	1101	0701	B3*0202	B4*01XX	0201	05XX	0202	0301
HD9	15	12	1501	1201	B3*0202	B5*0101	0102	-	0301	0602
HD10	1	17	0101	0301	B3*0101	-	0101	05XX	0201	0501
HD11	4	1	0401	0101	B4*01XX	-	0101	03XX	0302	0501
HD12	7	1	0701	0101	B4*01XX	-	0101	0201	0202	0501
HD13	13	4	1301	0401	B3*0101	B4*01XX	0103	03XX	0302	0603
HD14	7	16	0701	1601	B4*01XX	B5*0202	0102	0201	0202	0502
HD15	13	15	1301	1501	B3*0101	B5*0101	0102	0103	0602	0603
HD16	13	15	1302	1501	B3*0301	B5*0101	0102	-	0602	0604
HD17	1	16	0101	1601	B5*0202	-	0102	0101	0501	0502
HD18	1	17	0102	0301	B3*0202	-	05XX	0101	0201	0501
HD19	13	17	1303	0301	B3*0101	B3*0202	05XX	-	0301	0201
HD20	13	11	1301	1101	B3*0101	B3*0202	05XX	0103	0301	0603
HD21	4	-	0401	-	B4*01XX	-	03XX	-	0301	0302
HD22	13	16	1301	1601	B3*0202	B5*0202	0102	0103	0502	0603
HD23	4	17	0401	0301	B3*0202	B4*01XX	03XX	05XX	0301	0201
HD24	15	17	1501	0301	B3*0101	B5*0101	0102	05XX	0602	0201
HD25	7	15	0701	1501	B4*0103N	B5*0101	0102	0201	0303	0602
HD26	17	8	0301	0801	B3*0202	-	0401	05XX	0301	0402
HD27	11	15	1101	1501	B3*0202	B5*0101	0102	05XX	0301	0602
HD28	13	15	1301	1501	B3*0101	-	0102	0103	0603	-
HD29	11	7	1101	0701	B3*0202	B4*0103N	0201	05XX	0303	0301
HD30	17	10	0301	1001	B3*0101	-	0105	05XX	0201	0501
HD31	15	8	1501	0801	B5*0101	-	0102	0401	0602	0402
HD32	7	12	0701	1201	B3*0202	B4*01XX	0201	05XX	0202	0301
HD33	11	-	1101	-	B3*0202	-	05XX	-	0301	-
HD34	1	7	0101	0701	B4*01XX	-	0101	0201	0202	0501
HD35	17	12	0301	1201	B3*0101	B3*0202	05XX	-	0201	0301
HD36	11	7	1101	0701	B3*0202	B4*01XX	0201	05XX	0202	0301
HD37	13	11	1301	1101	B3*0101	B3*0202	0103	05XX	0603	0301
HD38	13	7	1301	0701	B3*0301	B4*01XX	0102	0201	0202	0604
HD39	15	7	1501	0701	B4*0103N	B5*0101	0102	0201	0602	0303
HD40	15	16	1501	1601	B5*0101	B5*0202	0102	-	0502	0602
HD41	1	4	0101	0401	B4*01XX	-	0101	03XX	0501	0302
HD42	4	16	0404	1601	B4*01XX	-	0102	03XX	0302	0502
HD43	4	12	0401	1201	B3*02	B4*01	*05	*03	0301	0302
HD44	4	17	0404	0301	-	-	-	-	0201	0302
HD45	4	17	0404	0301	B3*0101	B4*01	*03	*05	0201	0302
HD46	4	16	0403	1601	B4*01	B5*0202	0102	*05	0302	0502
HD47	4	16	0404	1601	B4*01	B5*0202	0102	*03	0302	0502
HD48	4	13	0401	1301	B3*0202	B4*01XX	0103	03XX	0301	0603
HD49	4	15	0401	1501	B4*01XX	B5*0101	0102	03XX	0302	0602

TABLE S2 HLA-DR and HLA-DQ alleles of all HD enrolled in cohort 2

Donor number	HLA serotype		HLA alleles							
	DR	DR	DRB1	DRB1	other DR	other DR	DQA1	DQA1	DQB1	DQB1
HD41	1	4	0101	0401	B4*01XX	-	0101	03XX	0501	0302
HD42	4	16	0404	1601	B4*01XX	-	0102	03XX	0302	0502
HD43	4	12	0401	1201	B3*02	B4*01	*05	*03	0301	0302
HD46	4	16	0403	1601	B4*01	B5*0202	0102	*05	0302	0502
HD47	4	16	0404	1601	B4*01	B5*0202	0102	*03	0302	0502
HD48	4	13	0401	1301	B3*0202	B4*01XX	0103	03XX	0301	0603
HD49	4	15	0401	1501	B4*01XX	B5*0101	0102	03XX	0302	0602
HD50	4	16	0402	1601	B4*01XX	B5*0202	0102	03XX	0302	0502
HD51	4	15	0404	1501	B4*01XX	B5*0101	0102	03XX	0302	0502
HD52	17	14	0301	1401	B3*0101	B3*0202	0104	05xx	0201	0503
HD53	17	11	0301	1101	B3*0101	B3*0202	05XX	-	0201	0301
HD54	7	15	0701	1501	B4*01XX	B5*0101	0102	201	0202	0602
HD55	11	13	1101	1301	B3*0101	B3*0202	0103	05XX	0301	0603
HD56	17	15	0301	1501	B3*0101	B5*0101	0102	05XX	0201	0602
HD57	7	14	0701	1401	B3*0202	B4*01XX	0104	201	0202	0503
HD58	8	11	0801	1104	B3*0202	-	0401	05XX	0301	0402
HD59	1	9	0101	0901	B4*0103	-	0101	03XX	0303	0501
HD60	17	14	0301	1401	B3*0101	B3*0202	0101	05XX	0201	0503
HD61	4	15	0401	1501	B4*01XX	B5*0101	0102	03XX	0302	0602
HD62	4	16	0401	1601	B4*01XX	B5*0202	0102	03XX	0302	0502
HD63	4	13	0401	1302	B3*0301	B4*01XX	0102	03XX	0302	0609
HD64	17	13	0301	1301	B3*0101	-	0103	05XX	0201	0603

TABLE S3 Frequency of HLA-DRB1 alleles in HD enrolled in cohort 1 and 2

HLA serotype	HLA allele	HD observed/n in cohort 1 (%)	HD observed/n in cohort 2 (%)
DR1		8 / 49 (16.3)	2 / 22 (9.1)
DR4		15 / 49 (30.6)	12 / 22 (54.6)
	DRB1*04:01	10 / 49 (20.4)	7 / 22 (31.8)
	DRB1*04:02	0 / 49 (0.0)	1 / 22 (4.5)
	DRB1*04:03	1 / 49 (2.0)	1 / 22 (4.5)
	DRB1*04:04	4 / 49 (8.2)	3 / 22 (13.6)
DR7		13 / 49 (26.5)	2 / 22 (9.1)
DR8		2 / 49 (4.1)	1 / 22 (4.6)
DR10		1 / 49 (2.0)	1 / 22 (4.6)
DR11		9 / 49 (18.4)	3 / 22 (13.6)
DR12		4 / 49 (8.2)	1 / 22 (4.6)
DR13		12 / 49 (24.5)	4 / 22 (18.2)
DR14		1 / 49 (2.0)	3 / 22 (13.6)
DR15		13 / 49 (26.5)	5 / 22 (22.7)
DR16		7 / 49 (14.3)	5 / 22 (22.7)
DR17		10 / 49 (20.4)	5 / 22 (22.7)

TABLE S4 Amino acid sequence and length of JCV peptides. The position of the each peptide is related to the respective protein of the JCV reference genome NC_001699. Peptides with single amino acid mutations are designated as variants (V1, V2, V3).

Peptide	Name and position	Sequence	Length (aa)
1	Agno (1-14)	MVLRQLSRKASVKV	14
2	Agno (10-25)	ASVKVSKTWSGTKKR	15
3	Agno (20-33)	GTKKRAQRILIFLL	14
4	Agno (25-39)	AQRILIFLLEFLLDF	15
5	Agno (35-49) V1	FLLDFCTGEDSVDGK	15
6	Agno (35-49) V2	FLLDFCTGEDRVDGK	15
7	Agno (43-57) V1	EDSVDGKKRQRHSGL	15
8	Agno (43-57) V2	EDRVDGKKRQRHSGL	15
9	Agno (53-65) V1	RHSGLTEQTYSAL	13
10	Agno (53-65) V2	RHSGLTEQRYSAL	13
11	Agno (53-65)	RHSGLQEQTYSAL	13
12	Agno (59-71) V1	EQTYSALPEPKAT	13
13	Agno (59-71) V2	EQRYSALPEPKAT	13
14	VP1 (1-14) V1	MAPTKRKGERKDPV	14
15	VP1 (1-14) V2	MAPTKRKGERHDPV	14
16	VP1 (8-22) V1	GERKDPVQVPKLLIR	15
17	VP1 (8-22) V2	GERHDPVQVPKLLIR	15
18	VP1 (17-31)	PKLLIRGGVEVLEVK	15
19	VP1 (26-40)	EVLEVKTGVDSITEV	15
20	VP1 (34-48)	VDSITEVECFLTPEM	15
21	VP1 (44-58) V1	LTPEMGDPDEHLRGF	15
22	VP1 (44-58) V2	LTPEMGDPNEHLRGF	15
23	VP1 (54-68)	HLRGFSKSISISDTF	15
24	VP1 (64-78) V1	ISDTFESDSPNRDML	15
25	VP1 (64-78) V2	ISDTFESDSPNFDML	15
26	VP1 (64-78) V3	ISDTFESDSPNKDML	15
27	VP1 (74-88) V1	NRDMLPCYSVARIPL	15
28	VP1 (74-88) V2	NFDMLPCYSVARIPL	15
29	VP1 (74-88) V3	NKDMLPCYSVARIPL	15
30	VP1 (81-95)	YSVARIPLPNLNEDL	15
31	VP1 (91-105)	LNEDLTCGNILMWEA	15
32	VP1 (101-115)	LMWEAVTLKTEVIGV	15
33	VP1 (108-122) V1	LKTEVIGVTSLMNVH	15
34	VP1 (108-122) V2	LKTEVIGVTTLMNVH	15
35	VP1 (108-122) V3	LKTEVIGVTALMNVH	15
36	VP1 (118-129) V1	LMNVHSNGQATH	12
37	VP1 (118-129) V2	LMNVHSNGQAAH	12
38	VP1 (118-129) V3	LMNVHSNGQASH	12
39	VP1 (123-137) V1	SNGQATHDNGAGKPV	15
40	VP1 (123-137) V2	SNGQAAHDNGAGKPV	15
41	VP1 (123-137) V3	SNGQASHDNGAGKPV	15
42	VP1 (133-147)	AGKPVQGTSFHFFSV	15
43	VP1 (143-157)	HFFSVGGEALELQGV	15
44	VP1 (151-165) V1	ALELQGVLFNYRTKY	15
45	VP1 (151-165) V2	ALELQGVLFNYRTTY	15
46	VP1 (161-175) V1	YRTKYPDGTIFPKNA	15
47	VP1 (161-175) V2	YRTTYPDGTIFPKNA	15
48	VP1 (161-175) V3	YRTTYPHGTIFPKNA	15
49	VP1 (171-186)	FPKNATVQSQVMNTEH	16

50	VP1 (182-196)	MNTEHKAYLDKNKAY	15
51	VP1 (193-208)	KNKAYPVECWVPDPTR	16
52	VP1 (203-217)	PDPTRNENTRYFGTL	15
53	VP1 (210-224)	NTRYFGTLTGGENVP	15
54	VP1 (220-234) V1	GENVPPVLHITNTAT	15
55	VP1 (220-234) V2	GENVPSVLHITNTAT	15
56	VP1 (220-234) V3	GENVPPVLHITKTAT	15
57	VP1 (229-243) V1	ITNTATTVLLDEFGV	15
58	VP1 (229-243) V2	ITKTATTVLLDEFGV	15
59	VP1 (239-253) V1	DEFGVGPLCKGDNLY	15
60	VP1 (239-253) V2	DEFGVRPLCKGDNLY	15
61	VP1 (249-263)	GDNLYLSAVDVCGMF	15
62	VP1 (259-273) V1	VCGMFTNRSGSQQWR	15
63	VP1 (259-273) V2	VCGMFTKRSGSQQWR	15
64	VP1 (259-273) V3	VCGMFTNRSGFQQWR	15
65	VP1 (259-273) V4	VCGMFTNRAGSQQWR	15
66	VP1 (259-273) V5	VCGMFTNGSGSQQWR	15
67	VP1 (269-283)	SQQWRGLSRYFKVQL	15
68	VP1 (270-283)	FQQWRGLSRYFKVQL	15
69	VP1 (279-294)	FKVQLRKRRVKNPYPI	16
70	VP1 (290-304)	NPYPISFLLTDLINR	15
71	VP1 (300-314)	DLINRRTPRVDGQPM	15
72	VP1 (310-321)	RVDDGQPMYGMDAQV	15
73	VP1 (319-331)	MAQVEEVRVFEGTE	14
74	VP1 (327-341) V1	FEGTEELPGDPDMMR	15
75	VP1 (327-341) V2	FEGTEQLPGDPDMMR	15
76	VP1 (335-349)	GDPDMMRYVDKYGQL	15
77	VP1 (341-354)	RYVDKYGQLQTKML	14
78	VP2 (1-15)	MGAALALLGDLVATV	15
79	VP2 (11-24)	LVATVSEAAAATGF	14
80	VP2 (20-34)	AATGFSVAEIAAGEA	15
81	VP2 (30-43)	AAGEAAATIEVEIA	14
82	VP2 (39-51)	EVEIASLATVEGI	13
83	VP2 (47-61)	TVEGITSTSEAIAAI	15
84	VP2 (57-71)	AIAAIGLTPETYAVI	15
85	VP2 (67-81)	TYAVITGAPGAVAGF	15
86	VP2 (77-88)	AVAGFAALVQTV	12
87	VP2 (84-97)	LVQTVTGGSAIAQL	14
88	VP2 (93-107)	AIAQLGYRFFADWDH	15
89	VP2 (103-115)	ADWDHKVSTVGLF	13
90	VP2 (111-125)	TVGLFQQPAMALQLF	15
91	VP2 (120-134)	MALQLFNPEDYYDIL	15
92	VP2 (130-142)	YYDILFPGVNAFV	13
93	VP2 (138-152)	VNAFVNNIHYLDPRH	15
94	VP2 (148-161)	LDPRHWGPSLFSTI	14
95	VP2 (157-170)	LFSTISQAFWNLVR	14
96	VP2 (166-181) V1	WNLVRDDLPALTSQEI	16
97	VP2 (166-181) V2	WNLVRDDLPSLTSQEI	16
98	VP2 (177-190)	TSQEIQRRTQKLFV	14
99	VP2 (185-197)	TQKLFVESLARFL	13
100	VP2 (193-205)	LARFLEETTWAIV	13
101	VP2 (201-215)	TWAIVNSPANLYNYI	15
102	VP2 (211-225)	LYNYISDYYSRLSPV	15
103	VP2 (221-234)	RLSPVRPSMVRQVA	14
104	VP2 (230-243)	VRQVAQREGTYISF	14
105	VP2 (239-251)	TYISFGHSYTQSI	13
106	VP2 (247-260) V1	YTQSIDDADSIQEV	14

107	VP2 (247-260) V2	YTQSIDNADSIQEV	14
108	VP2 (256-267)	SIQEVTQRLDLK	12
109	VP2 (263-277)	RLDLKTPNVQSGEFI	15
110	VP2 (273-286)	SGEFIERSIAPGGA	14
111	VP2 (282-296)	APGGANQRSAPQWML	15
112	VP2 (292-306)	PQWMLPLLLGLYGTV	15
113	VP2 (302-313)	LYGTVTPALEAY	12
114	VP2 (309-323)	ALEAYEDGPNKKKRR	15
115	VP2 (319-332)	KKKRRKEGPRASSK	14
116	VP2 (328-340)	RASSKTSYKRRSR	13
117	VP2 (332-344)	KTSYKRRSRSSRS	13
118	LTAg (1-14)	MDKVLNREESMELM	14
119	LTAg (10-24)	SMELMDLLGLDRSAW	15
120	LTAg (20-34)	DRSAWGNIPVMRKAY	15
121	LTAg (29-42)	VMRKAYLKKCKELH	14
122	LTAg (38-52)	CKELHPDKGGDEDKM	15
123	LTAg (48-62)	DEDKMKRMNFLYKKM	15
124	LTAg (56-70)	NFLYKKMEQGVKVAH	15
125	LTAg (63-77)	EQGVKVAHQPDFGTW	15
126	LTAg (73-85)	DFGTWNSSEVPTY	13
127	LTAg (81-94)	EVPTYGTDEWESWW	14
128	LTAg (90-105)	WESWWNTFNEKWDEDL	16
129	LTAg (99-113)	EKWDEDLFCHEEMFA	15
130	LTAg (109-123)	EEMFASDDENTGSQH	15
131	LTAg (119-133)	TGSQHSTPPKKKKKV	15
132	LTAg (129-143)	KKKKVEDPKDFPVDL	15
133	LTAg (138-152)	DFPVDLHAFLSQAVF	15
134	LTAg (148-162)	SQAVFSNRTVASFAV	15
135	LTAg (158-172)	ASFAVYTTKEKAQIL	15
136	LTAg (168-182)	KAQILYKKLMEKYSV	15
137	LTAg (176-190)	LMEKYSVTFISRHGF	15
138	LTAg (186-199)	SRHGFGGHNILFFL	14
139	LTAg (195-209)	ILFFLTPHRHRVSAI	15
140	LTAg (205-219)	RVSAINNYCQKLCTF	15
141	LTAg (215-229)	KLCTFSFLICKGVNK	15
142	LTAg (225-239)	KGVNKEYLFYSALCR	15
143	LTAg (235-249)	SALCRQPYAVVEESI	15
144	LTAg (244-258)	VVEESIQGGLKEHDF	15
145	LTAg (254-267)	KEHDFNPEEPEETK	14
146	LTAg (263-277)	PEETKQVSWKLVTQY	15
147	LTAg (273-287)	LVTQYALETKCEDVF	15
148	LTAg (282-296)	KCEDVFLLMGMYLDF	15
149	LTAg (291-305)	GMYLDFQENPQQCKK	15
150	LTAg (300-314)	PQQCKKCEKKDQPNH	15
151	LTAg (310-323)	DQPNHFNHHEKHYY	14
152	LTAg (319-332)	EKHYYNAQIFADSK	14
153	LTAg (328-342)	FADSKNQKSICQQAV	15
154	LTAg (338-351)	CQQAVDTVAAKQRV	14
155	LTAg (347-361)	AKQRVDSIHMTREEM	15
156	LTAg (357-370)	TREEMLVERFNFLL	14
157	LTAg (366-380)	FNFLLDKMDLIFGAH	15
158	LTAg (376-390)	IFGAHGNAVLEQYMA	15
159	LTAg (386-399)	EQYMAGVAWIHCLL	14
160	LTAg (395-409)	IHCLLPQMDTVIYDF	15
161	LTAg (405-419)	VIYDFLKCIVLNIPK	15
162	LTAg (415-429)	LNIPKKRYWLFKGPI	15
163	LTAg (425-439)	FKGPIDSGKTTLAAA	15

164	LTAg (435-449)	TLAAALLDLCGGKSL	15
165	LTAg (445-458)	GGKSLNVNMPLERL	14
166	LTAg (454-466)	PLERLNFELGVGI	13
167	LTAg (462-476)	LGVGIDQFMVVFEDV	15
168	LTAg (472-486)	VFEDVKGTGAESRDL	15
169	LTAg (482-495)	ESRDLPSGHGISNL	14
170	LTAg (491-506)	GISNLDCLRDYLDGSV	16
171	LTAg (500-514)	DYLDGSVKVNLERKH	15
172	LTAg (508-522)	VNLERKHQNKRTQVF	15
173	LTAg (518-532)	RTQVFPPGIVTMNEY	15
174	LTAg (528-542)	TMNEYSVPRTLQARF	15
175	LTAg (538-552)	LQARFVRQIDFRPKA	15
176	LTAg (548-563)	FRPKAYLRKSLSCSEY	16
177	LTAg (559-574)	SCSEYLLEKRILQSGM	16
178	LTAg (570-584)	LQSGMTLLLLLIWFR	15
179	LTAg (580-594)	LIWFRPVADFAAAIH	15
180	LTAg (590-604)	AAAIHERIVQWKERL	15
181	LTAg (600-613)	WKERLDLEISMYTF	14
182	LTAg (609-622)	SMYTFSTMKANVGM	14
183	LTAg (618-631)	ANVGMGRPILDFPR	14
184	LTAg (627-642)	LDFPREEDSEAEDSGH	16
185	LTAg (638-653) V1	EDSGHGSSTESQSQCF	16
186	LTAg (638-652) V2	EDSGHGSSTESQSQCS	16
187	LTAg (648-662) V1	SQSQCFSQVSEASGA	15
188	LTAg (648-662) V2	SQSQCSSQVSEASGA	15
189	LTAg (658-672) V1	EASGADTQENCTFHI	15
190	LTAg (658-672) V2	EASGADTQEHCTFHI	15
191	LTAg (668-681)	CTFHICKGFQCFKK	14
192	LTAg (674-688)	KGFQCFKKPKTPPPK	15
193	STAg (73-86)	DFGTWNSSEVGCDF	14
194	STAg (73-86)	DFGTWNSSEVSCDF	14
195	STAg (82-96) V1	VGCDFPPNSDTLYCK	15
196	STAg (82-96) V2	VSCDFPPNSDTLYCK	15
197	STAg (92-107)	TLYCKEWPNCATNPSV	16
198	STAg (103-117)	TNPSVHCPCLMCMLK	15
199	STAg (113-127)	MCMLKLRHRNRKFLR	15
200	STAg (123-137)	RKFLRSSPLVWIDCY	15
201	STAg (133-146)	WIDCYCFDCFRQWF	14
202	STAg (142-156)	FRQWFGCDLTQEALH	15
203	STAg (149-162)	DLTQEALHCWEKVL	14
204	STAg (158-172)	WEKVLGDTPYRDLKL	15

Abbreviations: Agno = agnoprotein; LTAg = large T antigen; STAg = small T antigen; VP = viral protein.

TABLE S5 Distribution of the peptides among the peptide pools used for epitope mapping. Peptides pools contain five peptides (except of peptide pool 2) and are related to the respective JCV protein. Peptide pools harboring peptide variants with single amino acid mutations are designated as variants.

Peptide pool	Peptides contained in the respective peptide pool					Protein
	Peptide 1	Peptide 2	Peptide 3	Peptide 4	Peptide 5	
1	1	2	3	4	5	Agno [a]
2	7	9	12			Agno [a]
3	6	8	10	11	13	Agno (variants) [b]
4	14	16	18	19	20	VP1 [c]
5	21	23	24	27	30	VP1 [c]
6	31	32	33	36	39	VP1 [c]
7	42	43	44	46	48	VP1 [c]
8	49	50	51	52	53	VP1 [c]
9	54	57	59	61	62	VP1 [c]
10	64	67	68	69	70	VP1 [c]
11	71	72	73	74	76	VP1 [c]
12	15	17	22	25	28	VP1 (variants) [d]
13	26	29	34	37	40	VP1 (variants) [d]
14	35	38	41	45	47	VP1 (variants) [d]
15	55	58	60	63	65	VP1 (variants) [d]
16	66	56	75	77	192	VP1 (variants) [d]
17	78	79	80	81	82	VP2 (N-VP2) [e]
18	83	84	85	86	87	VP2 (N-VP2) [e]
19	88	89	90	91	92	VP2 (VP2 / VP3) [f]
20	93	94	95	96	98	VP2 (C-VP2 / VP3) [g]
21	99	100	101	102	103	VP2 (C-VP2 / VP3) [g]
22	104	105	106	108	109	VP2 (C-VP2 / VP3) [g]
23	110	111	112	113	114	VP2 (C-VP2 / VP3) [g]
24	115	116	117	97	107	VP2 (C-VP2 / VP3) [g]
25	118	119	120	121	122	N-LTAg / N-STAg [h]
26	123	124	125	126	127	N-LTAg / N-STAg [h]
27	128	129	130	131	132	LTAg [i]
28	133	134	135	136	137	LTAg [i]
29	138	139	140	141	142	LTAg [i]
30	143	144	145	146	147	LTAg [i]
31	148	149	150	151	152	LTAg [i]
32	153	154	155	156	157	LTAg [i]
33	158	159	160	161	162	LTAg [i]
34	163	164	165	166	167	LTAg [i]
35	168	169	170	171	172	LTAg [i]
36	173	174	175	176	177	LTAg [i]
37	178	179	180	181	182	LTAg [i]
38	183	184	185	186	187	LTAg [i]
39	184	185	187	189	191	LTAg [i]
40	186	188	190	194	196	LTAg and STAg variants [j]
41	193	195	197	198	199	C-STAg [k]
42	200	201	202	203	204	C-STAg [k]

[a] Agno = peptides covering the complete agnoprotein sequence. [b] Agno (variants) = peptide variants harboring single amino acid substitutions compared to peptides of pools 1 and 2. [c] VP1 = peptides covering the complete agnoprotein sequence VP1 protein sequence. [d] VP1 variants = peptide variants harboring single amino acid substitutions compared to peptides of pools 4-11. [e] VP2 (N-VP2) = peptides covering the aminoterminal end of VP2 protein sequence. [f] VP2 (VP2 / VP3) = peptides covering VP2 only (peptides 88-90) or both VP2 and VP3 protein sequence (peptides 91 and 92). [g] VP2 (C-VP2 / VP3) = peptides covering the carboxyterminal end of VP2 protein, the sequence of which is identical to VP3 protein. [h] N-LTAg / N-STAg = peptides covering the aminoterminal end of large T antigen, the sequence of which is identical to the aminoterminal end of small T antigen. [i] LTAg = peptides covering the large T antigen sequence. [j] LTAg and STAg = peptide variants (harboring single amino acid substitutions compared to peptides of pools 38 and 41) derived from the large T antigen sequence and from the carboxyterminal small T antigen sequence. [k] C-STAg = peptides covering the carboxyterminal end of small T antigen.

TABLE S6 HLA-restricted prediction scores of JCV peptides identified as immunodominant by *in vitro* epitope mapping in the present study. The consensus method from the IEDB Analysis Resource was used for calculating the HLA-restricted prediction score of the immunodominant JCV peptides identified in the present study. The prediction score of the consensus method displays a percentile rank that combines the scores of four prediction methods (ARB, combinatorial library, SMM_align and Sturniolo) after comparison with five million random 15-mer peptides selected from SWISSPROT database (11). A percentile rank for each of the HLA-DR alleles found in HD of the present study was calculated. Top percentiles scores indicate good potential binders. The consensus method was shown to outperform other prediction (12, 13).

JCV peptide no.	JCV pool no.	JCV Protein and aa position	Percentile rank calculated with IEDB Analysis Resource															
			DRB1* 01:01	DRB1* 01:02	DRB1* 03:01	DRB1* 04:01	DRB1* 04:03	DRB1* 04:04	DRB1* 07:01	DRB1* 08:01	DRB1* 10:01	DRB1* 11:01	DRB1* 12:01	DRB1* 13:01	DRB1* 13:02	DRB1* 13:03	DRB1* 14:04	DRB1* 15:01	DRB1* 16:01
35	14	VP1 (108-122) V3	29.93	4.72	9.37	10.94	13.52	5.90	7.06	6.87	26.56	8.07	27.45	14.94	31.29	37.46	33.00	17.83	39.95
41	14	VP1 (123-137) V3	86.03	34.99	67.87	49.92	98.94	72.83	62.93	34.99	98.12	58.73	91.87	34.99	34.99	96.88	98.71	80.93	98.40
80	17	VP2 (20-34)	30.06	16.64	40.04	13.76	20.87	16.14	29.47	5.55	23.56	11.85	65.71	15.64	54.42	63.17	58.22	45.64	54.06
81	17	VP2 (30-43)	73.26	34.99	34.99	38.97	87.49	54.63	52.58	34.99	89.08	61.83	91.95	34.99	63.08	95.28	93.15	76.26	93.87
82	17	VP2 (39-51)	29.75	6.46	17.88	5.06	13.75	3.07	27.84	9.35	12.94	8.05	46.67	4.62	25.37	61.61	54.46	25.08	64.09
153	32	LTAg (328-342)	75.28	25.48	29.34	10.81	68.75	18.58	43.76	30.56	66.02	26.24	79.17	31.94	50.61	64.36	70.74	37.98	65.57
154	32	LTAg (338-351)	58.24	21.83	30.30	31.48	72.00	26.13	28.78	31.79	68.38	26.77	58.39	28.24	49.28	73.12	75.33	57.93	71.88
155	32	LTAg (347-361)	71.19	30.41	20.17	32.57	61.80	31.31	27.74	31.79	71.12	33.17	69.97	33.80	54.47	39.76	50.20	42.88	69.51
161	33	LTAg (405-419)	25.51	2.93	1.71	6.04	22.10	5.48	8.01	3.58	39.26	0.38	8.57	4.25	7.76	39.64	15.28	6.89	16.16
162	33	LTAg (415-429)	71.48	24.18	18.52	75.20	46.67	64.66	31.97	6.87	57.70	10.25	37.86	3.87	54.47	37.02	9.05	16.60	4.43

TABLE S7 Immunogenic BKV peptides reported in previous studies and the best matching JCV peptides used in the present study. Amino acids within the JCV peptides of the present study corresponding to sequences of immunogenic peptides as reported in previous studies are underlined. Recognition of immunogenic BKV peptides in the context of the respective HLA type is shown.

Immunogenic BKV peptides (as reported in previous studies)				Best matching JCV peptides (used in the present study)			
BKV protein and aa position	BKV peptide sequence	HLA binding	Reference	JCV peptide no.	JCV pool no.	JCV protein and aa position	JCV peptide sequence
BKV VP1 (44-52)	AITEVECFL	HLA-A*02:01	(10, 14)	20	4	JCV VP1 (34-48)	VDSITEVECFLTPEM
BKV VP1 (90-98)	STARIPLPNL	HLA-A*02:01	(9)	30	5	JCV VP1 (81-95)	YSVARIPLPNLNEDL
BKV VP1 (108-116)	LLMWEAVTV	HLA-A*02:01	(3, 14)	31	6	JCV VP1 (91-105)	LNEDLTCGNILMWEA
BKV VP1 (108-116)	LLMWEAVTV	HLA-A*02:01	(3)	32	6	JCV VP1 (101-115)	LMWEAVTLKTEVIGV
BKV LTAg (27-35)	LPLMRKAYL	HLA-B*07, HLA-B*08	(4)	120	25	JCV LTAg (20-34)	DRSAWGNIPVMRKAY
BKV LTAg (27-35)	LPLMRKAYL	HLA-B*07, HLA-B*08	(4)	121	25	JCV LTAg (29-42)	VMRKAYLKKCKELH
BKV LTAg (33-41)	AYLRKCKEF	HLA-A*24	(7)	121	25	JCV LTAg (29-42)	VMRKAYLKKCKELH
BKV LTAg (57-71)	TLYKKMEQDVKVAHQ	HLA-DRB1*03:01	(4)	124	26	JCV LTAg (56-70)	NFLYKKMEQGVKVAH
BKV LTAg (77-85)	WSSSEVPTY	HLA-A*01	(7)	126	26	JCV LTAg (73-85)	DFGTWNSSEVPTY
BKV LTAg (156-164)	RTLACFAVY	HLA-A*03	(7)	134	28	JCV LTAg (148-162)	SQAVFSNRTVASFAV
BKV LTAg (157-165)	TLACFAVYT	HLA-A*02:01	(5)	134	28	JCV LTAg (148-162)	SQAVFSNRTVASFAV
BKV LTAg (157-165)	TLACFAVYT	HLA-A*02:01	(5)	135	28	JCV LTAg (158-172)	ASFAVYTTKEKAQIL
BKV LTAg (159-167)	ACFAVYTTK	HLA-A*03	(7)	135	28	JCV LTAg (158-172)	ASFAVYTTKEKAQIL
BKV LTAg (172-180)	ILYKKLMEK	HLA-A*03	(7)	136	28	JCV LTAg (168-182)	KAQILYKKLMEKYSV
BKV LTAg (208-216)	SAINNFCQK	HLA-A*03	(7)	140	29	JCV LTAg (205-219)	RVSAINNYCQKLCTF
BKV LTAg (216-224)	KLCTFSFLI	HLA-A*02:01	(5)	141	29	JCV LTAg (215-219)	KLCTFSFLICKGVNK
BKV LTAg (222-230)	FLICKGVNK	HLA-A*03	(7)	141	29	JCV LTAg (215-219)	KLCTFSFLICKGVNK
BKV LTAg (227-235)	GVNKEYLLY	HLA-A*03	(7)	142	29	JCV LTAg (225-239)	KGVNKEYLFYSALCR
BKV LTAg (235-243)	YSALTRDPY	HLA-A*01	(7)	143	30	JCV LTAg (235-249)	SALCRQPYAVVEESI
BKV LTAg (242-250)	PYHTIEESI	HLA-A*24	(7)	143	30	JCV LTAg (235-249)	SALCRQPYAVVEESI

BKV LTAg (270-278)	VSWKLITEY	HLA-A*01	(7)	146	30	JCV LTAg (263-277)	PEETKQVSWKLVTQY
BKV LTAg (287-295)	VFLLLGMYL	HLA-A*24	(7)	148	31	JCV LTAg (282-296)	KCEDVFLLMGMYLDF
BKV LTAg (313-327)	PYHFKYHEKHFANAI	HLA-DRB1*01:01, HLA-DRB1*07:01	(6)	151	31	JCV LTAg (310-323)	DQPNHFNHHEKHYY
BKV LTAg (362-371)	MLTERFNHIL	HLA-A*02:01	(8, 14)	156	32	JCV LTAg (357-370)	TREEMLVERFNFLL
BKV LTAg (388-396)	QYMAGVAWL	HLA-A*24	(7)	159	33	JCV LTAg (386-399)	EQYMAGVAWIHCLL
BKV LTAg (394-402)	AWLHCLLPK	HLA-A*03	(7)	159	33	JCV LTAg (386-399)	EQYMAGVAWIHCLL
BKV LTAg (394-402)	AWLHCLLPK	HLA-A*03	(7)	159	33	JCV LTAg (386-399)	IHCLLPQMDTVIYDF
BKV LTAg (398-406)	CLLPKMDSV	HLA-A*02:01	(1)	160	33	JCV LTAg (395-409)	IHCLLPQMDTVIYDF
BKV LTAg (406-414)	VIFDFLHCI	HLA-A*02:01	(5, 8, 14)	161	33	JCV LTAg (405-419)	VIYDFLKCIVLNIPK
BKV LTAg (410-408)	FLHCIVFNV	HLA-A*02:01	(5, 8)	161	33	JCV LTAg (405-419)	VIYDFLKCIVLNIPK
BKV LTAg (414-422)	IVFNVPKRR	HLA-A*03	(7)	161	33	JCV LTAg (405-419)	VIYDFLKCIVLNIPK
BKV LTAg (414-422)	IVFNVPKRR	HLA-A*03	(7)	162	33	JCV LTAg (415-429)	LNIPKKRYWLFKGPI
BKV LTAg (422-430)	RYWLFKGPI	HLA-A*24	(7)	162	33	JCV LTAg (415-429)	LNIPKKRYWLFKGPI
BKV LTAg (472-480)	VVFEDVKGT	HLA-A*02:01	(10)	168	35	JCV LTAg (472-486)	VFEDVKGTGAESRDL
BKV LTAg (506-514)	SVKVNLEKK	HLA-A*03	(9)	171	35	JCV LTAg (500-514)	DYLDGSVKVNLERKH
BKV LTAg (553-567)	IYLRKSLQNSEFLLE	HLA-A*02:01	(8)	176	36	JCV LTAg (548-563)	FRPKAYLRKSLSCSEY
BKV LTAg (557-571)	KSLQNSEFLLEKRIL	HLA-A*02:01	(8)	177	36	JCV LTAg (559-574)	SCSEYLLEKRILQSGM
BKV LTAg (558-566)	SLQNSEFLL	HLA-A*02:01	(5)	176	36	JCV LTAg (548-563)	FRPKAYLRKSLSCSEY
BKV LTAg (558-566)	SLQNSEFLL	HLA-A*02:01	(5)	177	36	JCV LTAg (559-574)	SCSEYLLEKRILQSGM
BKV LTAg (579-587)	LLLIWFRPV	HLA-A*02:01	(5, 8, 14)	178	37	JCV LTAg (570-584)	LQSGMTLLLLLIWFR
BKV LTAg (579-587)	LLLIWFRPV	HLA-A*02:01	(5, 8)	179	37	JCV LTAg (580-594)	LIWFRPVADFAAAIH
BKV LTAg (604-612)	RLDSEISMY	HLA-A*01	(7)	181	37	JCV LTAg (600-613)	WKERLDLEISMYTF
BKV LTAg (613-627)	TFSRMKYNICMGKCI	HLA-A*02:01, HLA-DRB1*09:01	(4)	182	37	JCV LTAg (609-622)	SMYTFSTMKANVGM
BKV LTAg (613-627)	TFSRMKYNICMGKCI	HLA-A*02:01, HLA-DRB1*09:01	(4)	183	38	JCV LTAg (618-631)	ANVGMGRPILDFPR

TABLE S8 BKV- and JCV-crossreactive peptides reported in previous studies and compared to the best matching JCV peptides used in the present study. Amino acids, which are contained within the JCV peptides used in the present study and which show the sequence of the crossreactive JCV peptide, are underlined. Crossreactive peptide sequences are shown in the context of the respective HLA type.

BKV-encoded peptides crossreactive to JCV (reported in previous studies)				Best matching JCV peptides (used in the present study)			
BKV protein and aa position	BKV peptide sequence	HLA binding	Reference	JCV peptide no.	JCV pool no.	JCV protein and aa position	JCV peptide sequence
BKV VP1 (44-52)	AITEVECFL	HLA-A*02:01	(2, 10)	20	4	JCV VP1 (34-48)	VDSITEVECFLTPEM
BKV VP1 (108-116)	LLMWEAVTV	HLA-A*02:01	(3)	31	6	JCV VP1 (91-105)	LNEDLTCGNILMWEA
BKV VP1 (108-116)	LLMWEAVTV	HLA-A*02:01	(3)	32	6	JCV VP1 (101-115)	LMWEAVTLKTEVIGV
BKV LTAg (27-35)	LPLMRKAYL	HLA-B*07, HLA-B*08	(4)	120	25	JCV LTAg (20-34)	DRSAWGNIPVMRKAY
BKV LTAg (27-35)	LPLMRKAYL	HLA-B*07, HLA-B*08	(4)	121	25	JCV LTAg (29-42)	VMRKAYLKKCKELH
BKV LTAg (553-567)	IYLRKSLQNSEFLLE	HLA-A*02:01	(4)	176	36	JCV LTAg (548-563)	FRPKAYLRKSLSCSEY
BKV LTAg (557-571)	KSLQNSEFLLEKRIL	HLA-A*02:01	(4)	177	36	JCV LTAg (559-574)	SCSEYLLEKRILQSGM
BKV LTAg (613-627)	TFSRMKYNICMGKCI	HLA-A*02:01, HLA-DRB1*09:01	(4)	182	37	JCV LTAg (609-622)	SMYTFSTMKANVGM
BKV LTAg (613-627)	TFSRMKYNICMGKCI	HLA-A*02:01, HLA-DRB1*09:01	(4)	183	38	JCV LTAg (618-631)	ANVGMGRPILDFPR

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